

ANNALS OF BOTANY

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The Contents of the Vessels of *Fraxinus americana* L., with Respect to the Ascent of Sap

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With five Figures in the Text

INTRODUCTION

AMONG the outstanding problems in plant physiology those involved in the question of the ascent of water in the tree are of special and peculiar interest. Although these problems have been the subject of detailed investigations for many years, few decisive conclusions have been reached; and it would seem to be characteristic of existing data that they can be quoted in support of several conflicting views. It is not proposed here to give any account of the considerable amount of work which has already been performed—the type of experiment and many of the results will already be familiar, and a critical review has already been given (Priestley, 1935)—but rather to consider the results presented below strictly on their own merits, and to determine exactly what conclusions may safely be drawn from them. This course seems preferable, particularly in view of the ambiguity of the evidence available. Suffice it to say that the one definite point which may be taken for granted is that the so-called ‘root pressure’ is totally inadequate as a driving force. In the following pages, therefore, the force of root pressure is entirely disregarded.

In the case of the dicotyledonous tree, the nature of the vessel contents is obviously of primary importance in any consideration of the physical mechanism of the ascent of sap. It is fundamental to the Cohesion Theory that these vessels should contain continuous columns of water under rather high tensions (Dixon and Joly, 1895; Askenasy, 1895). That these continuous columns could continue to operate throughout a whole growing season may at least be questioned, particularly in such trees as the ring-porous dicotyledons, whose vessels, as shown by recent work at Leeds (Priestley, 1935), run as probably uninterrupted columns from root to crown. The breaking of such columns would undoubtedly lead to the formation of bubbles of water vapour (partial vacua above water columns) into which gases would diffuse from the atmosphere via the wet vessel walls. During this penetration of gases the water below the partial vacua will be withdrawn into the still functioning conducting

system and we may expect, in time, a considerable length of vessel to be free of liquid water. Under such circumstances the vessel will almost certainly be functionless as a conductor of water and serve, in future, only as a reservoir. It is therefore possible to apply a crucial test of the Cohesion Theory, to determine whether the vessels contain continuous liquid columns or merely gas under reduced pressure. It is from this point of view that the following investigation was attempted.

The fact that the wood of a ring-porous tree, when cut under coloured liquids, will inject readily for considerable lengths has been known for some time, and would seem at first sight to be conclusive. Such injection is most obviously noticed at the commencement of the growing season, when the new vessels are lying on the surface of the old wood, and can readily be demonstrated when the bark is removed. Since water is virtually incompressible, it might seem that the fact of injection rules out the possibility of the existence of continuous water columns. Detailed consideration of the facts, however, shows that on the basis of such experiments alone there is no unique explanation of injection; rather are there several possible alternatives, none of which can be ruled out *a priori*. Since we propose to examine the findings of the present research without consideration of other evidence, the various possibilities assume particular importance. Certainly we can say immediately that the force of surface tension is entirely inadequate (surface effects would cause water in a capillary 0.1 mm. radius to rise only about 15 cm.); nor, on the other hand, can the liquid be considered as travelling along a transpiration stream, for the liquid moves both upwards and downwards from the cut surface. Again, the possibility that water is being withdrawn from the cut vessel into neighbouring, intact vessels may also be ruled out, since a series of neighbouring vessels may be injected one after another. Nevertheless, there would seem to remain three possible explanations, which may be enumerated as follows:

1. The liquid is moving, both upwards and downwards, into a partial vacuum under the influence of the pressure of the atmosphere. We should expect the initial rate of injection to be approximately the same in both directions, unless the injection is made below the water-level in the vessel, when upward injection should take place much more rapidly than downward.

2. The liquid is moving into vessels occupied by continuous columns which are being withdrawn rapidly into the parenchyma of root, stem, and leaf. Prior to injection the water available to these cells was under rather high tensions, and injection places at their disposal what we may perhaps call 'free' water. Immediately the cut is made tensions are released in the water columns, except such as arise from the weight of the column, and this water, now under low tension, may be withdrawn into the living cells.

3. Injection is due to the fact that, under high tensions in a continuous liquid column, the vessel is contracted to some extent. Immediately these

tensions are released by placing the columns in direct communication with a supply of water under atmospheric pressure, the vessel expands and draws in the injecting fluid. It can be shown, however, that a tree only thirty feet high will inject to a height of twenty feet, while the contraction of the vessel may be only of the order of a few per cent. (Bode, 1923). In order to explain this observed length of injection in terms of elastic expansion, then, it is necessary to postulate a considerable degree of anastomosis in the vessel system. Although in trees like *Fraxinus* anatomical evidence would suggest that the degree of anastomosis is insufficient (Handley, 1936), it seems better for the moment to assume that this third condition is possible, at least for the initial stages of injection.

It is convenient, at this point, to emphasize the disadvantage inherent in the present type of experiment—a point which has been made several times in the literature. In cutting into the vessel system we are introducing a new factor, and any results obtained refer primarily to the vessel *after* injection, with possibly no significance for the state of affairs *before* injection. If, for instance, we find that injection is to be explained along the lines laid down in paragraph 1 above, then the partial vacuum may have existed in the intact vessel, or it may have been initiated by a breaking of a water column consequent upon the insertion of the knife blade. Consideration of the numerical results presented below tends to show that this type of error is not a serious factor in the present problem. At the same time, it must be clearly recognized that the rate at which the injecting liquid enters the vessel bears no relation to the movement (if any) in the vessel before injection.

As will be shown below, it is often possible to determine which of the explanations holds in actual practice by measuring rates of injection. From such measurements, using formulae corresponding to theoretical considerations of the various possibilities, a series of simple calculations may be made which enable each alternative to be tested separately. Further consideration of such tests is best postponed until a more complete analysis of the problem has been made.

The present method of attack, then, consists of a series of measurements of velocities and vessel diameters, and calculation of pressures. It is shown that, in the trees under investigation, many of the vessels probably contain gas under reduced pressure, while others contain continuous liquid columns, and that in those vessels which contain liquid columns the initial injection, at any rate, is due to elastic expansion. The research was commenced in the spring of 1934, though imperfection of technique made it impossible to arrive at any definite conclusions at that time. It is not proposed to give any detailed account of these earlier experiments, since apparatus which was placed at the author's disposal at Cornell University, U.S.A., in 1936, has enabled much more precise determinations to be made. Nevertheless, in so far as the earlier work supports and extends the later, it is necessary to make brief mention of it.

EXPERIMENTAL METHODS

The trees used in the following investigation were four well-grown specimens of *Fraxinus americana*, some 10 to 12 metres high and some 30 cm. diameter at breast height, in the vicinity of Ithaca, N.Y., U.S.A. Observations were made during late spring, in the late morning or early afternoon of bright, clear days. In order to make the wood available for experiment, a panel of bark was removed from the bole of the tree, care being taken that the incision along the upper and lower edges of the panel did not penetrate the wood below. A plasticine cup was attached to the lower end of the panel, the injecting liquid was poured in, and the wood cut below its surface. Fig. 1 will make the arrangement clear. In the earlier experiments observations were made over a range of 3 or 4 ft., whereas in later work, when a motion-picture camera was used as a timing device, it was impossible to use panels with dimensions greater than 20 to 30 cm. It is in this respect that the earlier work supplements the later. The injecting liquid used was Indian ink diluted to ten times its volume with distilled water. Such a suspension has the advantage of being aqueous, so that it wets the walls of the vessels and cannot pass end walls; and although there is some danger of blocking the vessels by infiltration, only in exceptional cases were signs of this observed in the brief duration of the experiments. Indian ink possesses the further advantage of showing strong contrast with the white wood of the tree, presenting a very suitable medium for photography. The ink was boiled for a short time prior to each experiment, to avoid production of gas bubbles from the injecting liquid itself, and was kept in filled, tightly stoppered bottles until used.

Before it was realized how rapidly injection takes place, attempts were made to measure velocities by means of a stop-watch, observing the time taken for the meniscus to travel a measured distance. It has since been shown that the initial rate of injection may be as high as 100 cm. per sec., so that a stop-watch measuring to $\frac{1}{2}$ sec. is obviously useless in measuring rates of movement over a few centimetres. A second method of timing tried was a modification of that used by Galileo, using the rate of loss of water from calibrated burettes. With the help of four observers, this method proved successful, except for the measurement of initial rates of injection. The results were sufficiently interesting to encourage further research.

The disadvantage of using the motion-picture camera in this type of observation is, of course, that, since each series must be a 'close-up' in order that individual vessels may subsequently be observed on the film, the panel cannot be longer than 20 to 30 cm. ('panoraming' is difficult at this short distance, since it necessitates continuous and accurate change of focus). The observations are made, therefore, on a comparatively short length of trunk and are in no sense comprehensive. As will be shown below, however, these initial velocities are often critical in determining the nature of the vessel

contents, although longer range determinations are also useful. The combination of both types of timing apparatus is a subject for further research.

In order to ensure accurate timing, and as a check on the speed indicator of the camera, a seconds pendulum was set up alongside the panel and in the field of view of the camera. This was set swinging at small amplitude, the camera was set in motion, and injection begun a few seconds later. A sharp scalpel with the blade held vertically, rather than a needle, was used in making the incision, since the orifice so made was larger (and therefore the error in the first measurements smaller) and it was still possible to inject single vessels. A series of vessels, spaced 1 cm. apart, were thus injected across the panel. At the end of the experiment a metre rule was held against the wood in order to calibrate the film for length measurement and the vessels injected were numbered on the tree in Indian ink. Measurements of vessel diameter were made with an eyepiece micrometer, from material stored in dilute glycerine-alcohol, and each measurement could thus often be referred to the corresponding injection on the film.

The camera used was a Kodak Special K model for 16 mm. film. Du Pont Positive film was found to be the most suitable of those tried, having a very small grain, and was developed in the Plant Physiology Laboratories at Cornell University. Detailed observation of the film showed that its rate of motion was perfectly steady for

each observation. If, then, as is usual in the experiments described below, the

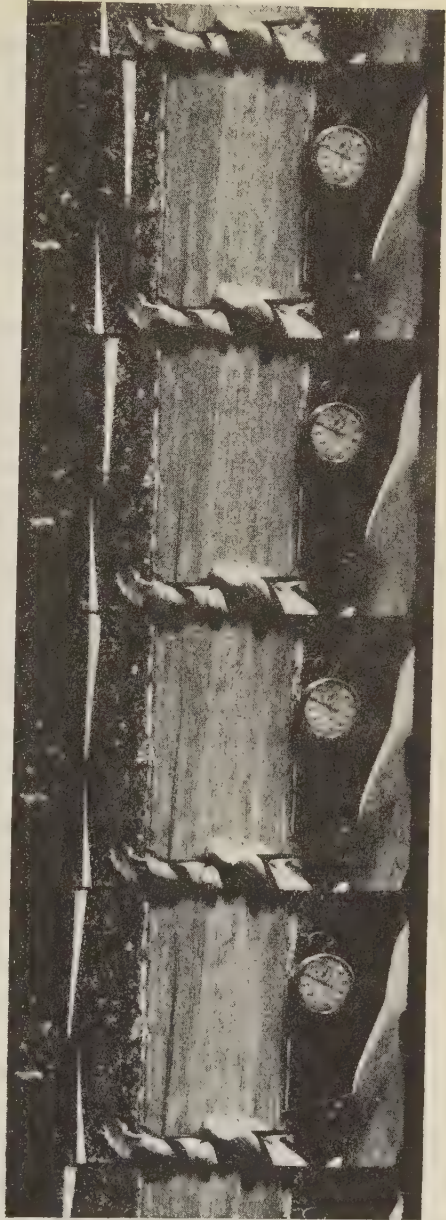


FIG. 1. A strip of film showing the injection of two vessels. Notice the positions of the pendulum on the left of the panel. Time interval between consecutive frames, 0.056 sec.

film was passing the lens system at a rate of 16 frames per sec., we may safely say that the interval between two consecutive frames is 0.056 second. By observing the position of the ink meniscus, blurred very slightly on the film owing to the small, though appreciable, exposure time, a curve may thus be constructed relating the length of the ink column to the time, and the velocities at different heights thus determined. In Fig. 1 is presented a series of four consecutive frames from an actual experiment, showing part of an injection. In this particular experiment a stop-watch was also included as a check on the pendulum.

TREATMENT OF DATA

From the velocities thus calculated and the heights of the meniscus to which they refer, it is now possible to make certain deductions concerning the alternatives mentioned on p. 2. As the calculations and the assumptions underlying them are different for each of the three possibilities, it is preferable to treat them separately. The one assumption common to all three is that Poiseuille's Law may be taken to hold over at least short distances. This law states that, for steady flow of a liquid, viscosity η , along a capillary of r cm. radius and l cm. length under a pressure difference P dynes between the two ends of the capillary tube,

$$P = \frac{8l\eta Q}{\pi r^4} = \frac{8\eta}{r^2} lv, \quad (1)$$

where Q is the volume of liquid passing down the tube per second and v is the corresponding velocity.

The essential conditions for which the law represents capillary flow are:

1. The capillary must have smooth walls. In the case of the vessels this is approximately true, for the irregularities due to pitting are too slight to have any appreciable effect.

2. The liquid layer in contact with the walls must be stationary. Since the injecting fluid (and the liquid content of the vessels, if any) wets the vessel walls, this condition is probably fulfilled equally as well as in the case of water flowing in glass capillaries.

3. The quantity v must be less than $\frac{1000\eta}{r}$, for at this point flow becomes turbulent and no formulae apply. It will be obvious from the results quoted below that this condition holds in the present case.

4. The acceleration (or deceleration) of the liquid must correspond to a negligible force. This is probably true in the present case, although accurate measures of this quantity are impossible. Rough calculations show, however, that the correction to be applied is negligible.

5. The capillary must have uniform circular cross-section. The vessel diameter is certainly not uniform throughout the length of trunk investigated and may vary by as much as 3 per cent. Vessel diameters were measured at

several points along the injection, and the average of these inserted in the formula. Neither, however, is the vessel quite circular in section, but rather approaches the elliptical. The modification of Poiseuille's Law for flow through elliptical tubes may be readily deduced as

$$P = 4\eta lv \frac{a^2 + b^2}{a^2 b^2}, \quad (2)$$

where a is the major, and b the minor axis of the ellipse. The ratio of the pressures calculated according to the equations (1) and (2) is therefore

$$\frac{P_1 \text{ (elliptical bore)}}{P_2 \text{ (circular bore)}} = \frac{a^2 + b^2}{a^2 b^2} \cdot \frac{r^2}{2}.$$

If the elliptical and circular tubes have the same area of cross-section, then

$$r^2 = ab \quad (3)$$

and

$$\frac{P_1}{P_2} = \frac{a^2 + b^2}{2ab}.$$

In the present investigation only those vessels were recorded for which $a/b \leq 1.3$. In this case $P_1/P_2 \leq 1.03$, and there is a maximum error of the order of only 3 per cent. in using formula (1) in conjunction with (3) instead of (2). On account of its more ready manipulation in routine calculations, formula (1) was therefore invariably used.

One further point in connexion with the use of Poiseuille's Law must be mentioned. In the present case the length of capillary under observation includes the meniscus; and difficulties of a mathematical nature make it impossible to take any account of the 'end effect' thus introduced, in consideration of vessels with gas content.

Let us now re-examine the three possible explanations of injection in terms of these formulae.

1. If the vessel is filled with gas under reduced pressure then we may express the pressure difference between the cut end and the meniscus at any time as

$$\pi - P_t = \frac{8\eta}{r^2} lv,$$

where π is the pressure of the atmosphere, and P_t is the pressure in the vessel at time t , l being the length of the vessel occupied by the injecting fluid. Now it is immediately obvious that in this case

$$P_t > 0. \quad (4)$$

In addition we may note that the length of the vessel over which observations are made is small compared with the total vessel length. Hence P is constant and therefore

$$lv = \text{constant}. \quad (5)$$

If the injection is such that (4) is not satisfied then the vessel contains something other than gas or vapour; while if both are found to apply within the

limits of experimental error we may reasonably assume gaseous content. Failure to satisfy (5) alone may be explained either by the presence of a continuous liquid column or by a vessel length comparable with the length injected.

2. If, on the other hand, a continuous liquid column is being raised at velocity v , then the length l in the formula is now equivalent to the height of the tree at least (for no account is taken of cross walls). If the tension in the water column be T , then

$$\pi + T - gL = \frac{8\eta}{r^2}Lv,$$

where g is the acceleration due to gravity, and L the vessel length.

In order to absorb water from the system at this observed rate the osmotic pressure of the receiving cells must be greater than T . We may assume that the osmotic pressure of the mesophyll is seldom greater than twenty atmospheres (Korstain, 1924), so that if T , calculated according to this formula, is much higher than this figure, we may safely assume that a continuous liquid column is not moving into the leaf.

3. The case of elastic expansion of a vessel presents a much more complicated problem. Theoretical consideration of a similar case has been attempted by Frey-Wyssling (1933), working on the latex ducts of *Hevea brasiliensis*. The general case of flow into an elastic capillary system leads to an insoluble differential equation. The solution of a case only approximating to reality presents considerable difficulties, and it seems better for present purposes to solve a purely hypothetical case by legitimate methods rather than to attempt a solution of a more nearly analogous problem by invalid assumptions.

In the general case the contour of the vessel immediately after injection may be expected to appear as in Fig. 2. Let us consider the case illustrated in Fig. 3. We will suppose that at any time the part AB of the vessel injected has a uniform radius R , while the remainder of the vessel, which we are assuming full of water under tension, has a smaller radius a constant along the length of the vessel at any given time but increasing with time. The observed increase in diameter of injected vessels is so small that these assumptions, though giving the wrong wall contour, will probably yield results of the correct order.

Under these conditions the rate of transfer of liquid through the tube at any time t may be represented by

$$\frac{dQ}{dt} = \frac{\pi R^4}{8\eta x} \cdot T \quad (7)$$

and, if the velocity of the meniscus be dx/dt , then

$$\frac{dQ}{dt} = \pi R^2 \frac{dx}{dt}, \quad (8)$$

i.e. the rate of passage of the liquid is equal to the rate of increase in the volume of vessel injected.

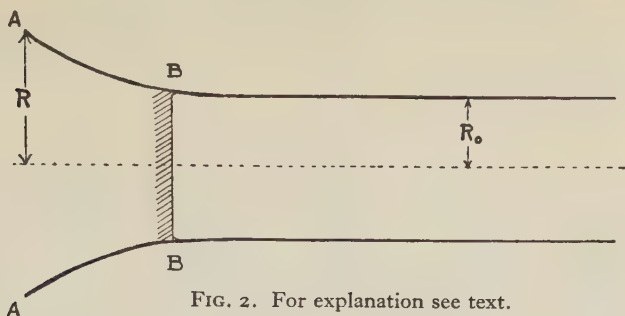


FIG. 2. For explanation see text.

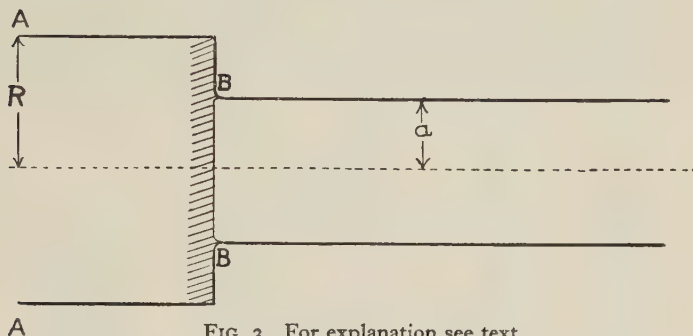


FIG. 3. For explanation see text.

Further, since the whole of the vessel above the meniscus is filled with water, none of which is allowed to escape, we have

$$\pi a^2(L-x) = \text{constant} = C, \quad (9)$$

and finally, for elastic expansion of the vessel we have, according to Hooke's Law,

$$a = R - mT \quad (10)$$

where m is a constant.

Substituting (10) in (7), we have:

$$\frac{dQ}{dt} = \frac{\pi R^4}{8\eta x} \frac{(R-a)}{m}, \quad (11)$$

whence, by substituting dx/dt from (9) into (8), equating (8) and (11) and integrating we get

$$R-a = Ae^{-\frac{\pi R^5}{k(L\pi R^2 - C)} \cdot t},$$

where A is a constant and

$$k = \frac{16Cm\eta}{\pi R^2}.$$

Hence, from (11),

$$x \frac{dx}{dt} = A \cdot \frac{R^2}{8m\eta} e^{-\frac{\pi R^5}{k(L\pi R^2 - C)} \cdot t}.$$

The product $x(dx/dt)$ (or lv) should therefore decrease logarithmically with time.

If, then, we find lv to behave in this way, and the value for P to be such as to rule out the other possibilities, we may assume that injection occurs as a result of such elastic expansion.

RESULTS

In presenting the experimental results it has been considered advisable to tabulate them according to their probable significance in view of the above reasoning. Tables I and II therefore correspond to cases for which 'gas content' and 'water content' respectively are probable, and Table III includes the uncertain data. The experimental errors at the head of the sixth column of each table, calculated from the errors in reading l and v , are, of course, only approximate, since the exact errors vary with the height of the meniscus. We are here, however, concerned chiefly with the order of the pressures

TABLE I
Gas Content

Ash I and II were approximately 10 metres, and Ash III 12 metres high.

Specimen.	Diameter (mm.).	Velocity v (cm./sec.).	Height l (cm.).	lv .	Gas pressure in vessel (atm.) (± 0.01).	Water tension in vessel (atm.).
<i>Ash I</i>						
vessel 1		129.0	4.2	542	0.61	113
		64.0	10.6	680	0.51	
		40.0	13.6	544	0.61	
2	0.210	59.6	8.7	522	0.68	50
		42.0	12.9	544	0.71	
		32.5	16.3	530	0.69	
3	0.144	37.6	5.6	209	0.68	14
		10.0	8.9	89	0.86	
		9.6	10.0	96	0.85	
		7.7	12.5	96	0.85	
		7.4	14.0	103	0.84	
		5.6	15.2	85	0.87	
11	0.184	84.0	2.9	242	0.77	78
		46.0	9.4	432	0.58	
		40.0	12.2	490	0.53	
		36.0	14.2	512	0.51	
		28.0	15.8	443	0.58	
12	0.181	99.0	2.9	285	0.72	103
		60.0	7.5	450	0.58	
		46.0	10.5	485	0.54	
		27.0	12.7	342	0.68	
		27.0	14.3	386	0.62	
		28.0	15.8	444	0.58	
13	0.210	43.0	8.0	344	0.74	34
		44.0	10.5	464	0.65	
		22.0	13.1	287	0.78	
		20.0	14.9	298	0.77	

TABLE I (contd.)

Specimen.	Diameter (mm.).	Velocity, <i>v</i> (cm./sec.).	Height, <i>l</i> (cm.).	<i>lv.</i>	Gas pressure in vessel (atm.) (± 0.01).	Water tension in vessel (atm.).
<i>Ash II</i>						
vessel 2	0.240	34.0	6.3	216	0.87	24
		22.0	9.4	207	0.88	
		20.0	11.7	234	0.86	
		15.0	13.8	207	0.88	
		17.0	15.6	265	0.84	
		12.0	18.6	223	0.87	
3	0.240	58.0	3.95	229	0.87	41
		13.1	5.45	71	0.96	
		18.4	6.90	130	0.92	
<i>Ash III</i>						
vessel 2	0.212	55.0	4.6	253	0.81	41
		36.0	7.0	252	0.81	
		21.0	9.0	189	0.85	
		13.0	11.0	143	0.89	
		8.0	13.0	104	0.92	
3	0.246	56.0	8.1	453	0.75	31
		42.0	10.8	455	0.75	
8	0.242	37.0	6.0	222	0.88	24
		32.0	8.0	256	0.86	
		28.0	10.0	280	0.85	
		23.0	12.0	276	0.87	
		17.0	14.0	238	0.88	
		13.0	16.0	208	0.89	
		10.0	18.0	180	0.90	

calculated rather than unnecessarily precise details, and no useful purpose would be served by tabulating the experimental errors. In Fig. 4 is presented a typical series of readings of the height of the meniscus, plotted as a graph against time; this is included merely to indicate the degree of accuracy with which the experimental data allow the determination of velocities, according to the method already mentioned (p. 6).

It will be clear in Table I that the product *lv*, and hence the pressure within the vessel, is approximately constant for each vessel with the almost invariable exception of the first determination, and of one or two later determinations. With the exception of Ash I, vessel 3, the tension in a liquid column supposed to be moving into the leaf appears to be so high as to rule out any possibility of such a state of affairs.

The gas pressure inside the vessel never falls below zero, so that on the basis of the criteria mentioned above there can be little doubt but that the vessels listed here contain only gas, at pressures ranging from 0.4 to 0.9 atmosphere. The discrepancy in the first readings in a series may be ascribed to one of two possible causes. Either the size of the incision is controlling flow at the initial stages or, more probably, the measurement was taken before the knife was completely withdrawn (it is often difficult to see exactly when

the withdrawal takes place). One case needs particular mention. In Ash III, vessel 2, the product lv decreases with increasing height of injection, and yet does not fit an exponential curve. An explanation, already briefly mentioned,

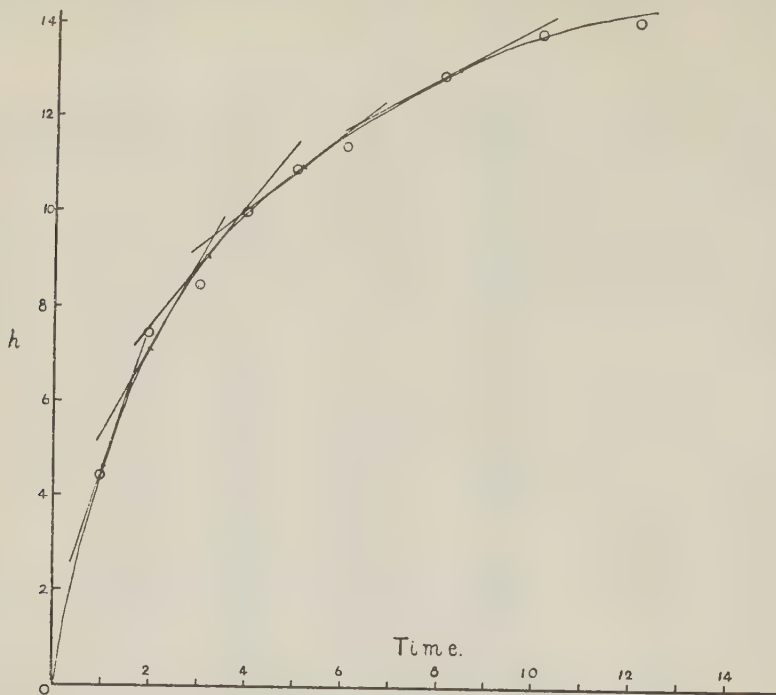


FIG. 4. A graph showing the relation between the height of the ink meniscus and the time for Ash III, vessel 2, illustrating the method used in determining velocities.

immediately offers itself. Assuming gas content, the product lv can be constant only so long as the vessel is long compared with the injection distance observed. If, however, the distance between the vessel termination and the cut is rather short, then the pressure in the vessel will vary according to the equation

$$P = P_0 \frac{L}{L-l},$$

where L is the distance referred to and P_0 is the pressure inside the vessel before the cut is made.

Hence

$$P_1(L-l_1) = P_2(L-l_2) \quad (= P_0L),$$

so that if $l_2 > l_1$, then $P_1 < P_2$ and therefore $l_1v_1 > l_2v_2$.

Further, the length L may thus be determined from pairs of pressure determinations. Such a measure of 'vessel length' is hardly exact, since the experimental error of the pressure determinations is comparable with the pressure differences. It may be calculated that the length L is 58 cm. (with limits of 114 cm. and 40 cm.).

Among the results in Table II are presented four vessels from Ash I to which gas content hypothesis clearly does not apply. The (hypothetical) gas pressures fall well below zero and the product lv is not constant. In the case of Vessel 15 the measured velocities are too erratic to allow the application

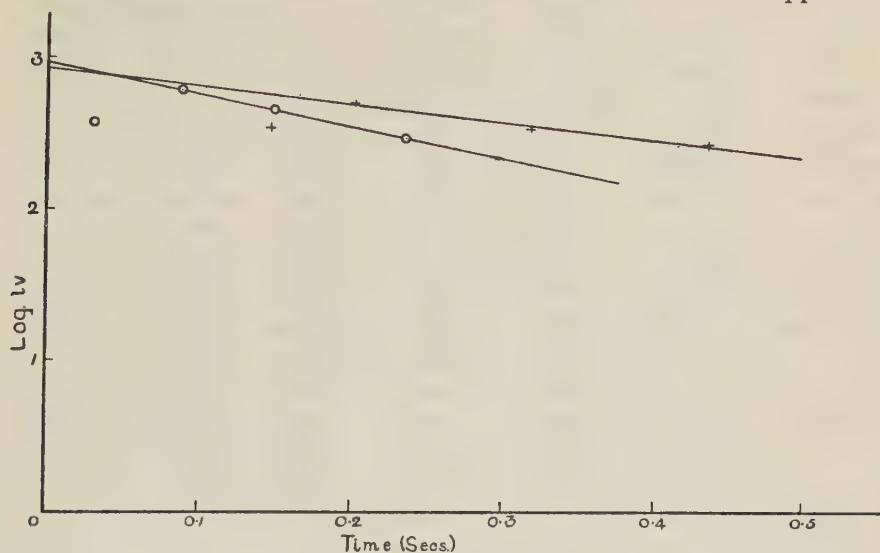


FIG. 5. Ash I, vessel 5: $\log lv = 2.95 - 2t$. Ash I, vessel 14: $\log lv = 2.9 - (2.7)t$.

of any theoretical considerations; a variation which is possibly due to a sticking of the meniscus or to a temporary blocking of the vessel by infiltration. Of the remaining vessels, the two for which a sufficient number of determinations was possible show a logarithmic decrease of lv . This will be clear from Fig. 5. On the basis of the hypothetical case of elastic capillary flow discussed above, we may assume that these vessels contain continuous water columns and that injection results from an expansion of the vessels, consequent upon the release of tensions. We may further assume, as a first approximation, that since

$$lv = Be^{-Kt},$$

then

$$P = \frac{8\eta}{a^2} Be^{-Kt} = P_0 e^{-Kt},$$

where P is the effective pressure forcing the ink column into the vessel, i.e. the pressure difference between the external atmosphere and the meniscus, and P_0 is the pressure at time $t = 0$.

P_0 appears to be of the order of 3 atmospheres and represents, very approximately, the tension in the vessel at the point of cutting immediately after the incision is made (atmospheric pressure may be neglected since the tree is approximately 40 ft. high). It is questionable whether this may be taken to

Preston—The Contents of the Vessels of

TABLE II

Water Content

Ash I was 10 metres, and Ash IV 12 metres high

Specimen.	Diameter (mm.).	Velocity, <i>v</i> (cm./sec.).	Height, <i>l</i> (cm.).	<i>lv.</i>	Gas pressure in vessel (atm.) (±0.01).	Water tension in vessel (atm.).
<i>Ash I</i>						
vessel 5	0.110	114.0	3.2	365	—0.14	356*
		73.0	8.4	617	—1.10	
		42.0	11.6	490	—0.90	
		24.0	14.1	338	0.07	
9	0.136	51.0	8.7	446	0.23	89
		49.0	12.7	620	—0.10	
14	0.100	90.0	4.1	374	—0.28	306†
		61.0	8.5	520	—0.78	
		27.0	13.6	346	—0.25	
		20.0	14.9	298	—0.04	
15	0.138	99.0	2.9	284	0.38	164
		82.0	8.1	670	—0.14	
		19.0	12.2	228	0.62	
		31.0	16.4	485	0.19	
<i>Ash IV</i>						
vessel 1	0.222	<i>Upper</i>	e.g.			
		20 (const.)	8.0	160	0.90	13
		<i>Lower</i>				
		18.2	0.51			
		0.0	1.02			
		3.6	1.50			
2	0.186	<i>Upper</i>	e.g.			
		5.6 (const.)	2.0	11.2	0.99	5.2
		<i>Lower</i>				
		12.2	0.5			
		12.2	1.5			
		12.2	2.7			
3	0.230	21.0	3.3			
		<i>Upper</i>	e.g.			
		12 (const.)	4.0	48.0	0.97	7.2
		<i>Lower</i>				
		3.9	1.67			
		18.1	3.0			

* Fits the exponential curve Tension = $3e^{-(2.0)t}$ † Fits the exponential curve Tension = $3e^{-(2.7)t}$

represent the tension in the vessel system before cutting into it, since the equations are not valid before time $t = 0$. At any rate, however, the figures give no evidence for very high tensions in the continuous liquid columns.

The three determinations on Ash IV may be interpreted along quite different lines. This particular tree behaved quite differently from any other

investigated. It was customary, before commencing each series of determinations on a tree, to inject at a point half-way up the panel in order to be sure that the injection occurred both upwards and downwards. Ash IV was the only tree observed for which the initial rate of upward injection was appreciably different from the downward rate. Accordingly, the panel on this tree was injected not at the bottom, but half-way up, and a series of determinations made on both injection directions. A plasticine cup could now, of course, not be used, and the ink was held on the point of the scalpel. Only those determinations listed could later be definitely connected with vessels in the preserved trunk specimens. In each case the upward velocity is constant. The downward rate in vessel 1 is initially about the same as the upward rate and then falls off; in vessel 3 the downward rate is lower than the upward but rises steeply; and in vessel 2 the downward rate is appreciably higher than the upward and tends to increase. On the gas content hypothesis, the constant upward rate of injection is understandable only on the assumption that the cut is made well below the water-level; and, if that were true, then we should expect very slow downward injection, if any. Hence this hypothesis would appear to be ruled out. The only possible explanation seems to be that a continuous liquid column is moving, upwards and downwards, and it is important in this regard to notice that the water tensions calculated on this assumption are the lowest recorded in these investigations. Even this conclusion may be questioned since, according to the results presented in Table II, these tensions are probably sufficiently appreciable to contract the vessel and should lead to injection by elastic expansion. No figures can be presented for the downward injection, of course, in view of our lack of knowledge as to the extent of the root system. On the basis of these calculations alone, therefore, we may conclude that we have here continuous columns moving up into the leaves and down to the roots; though the question as to the sink into which the column is moving, so often asked concerning upward injection, is even more difficult to answer for the downward injection.

Among those observations tabulated as questionable several are of particular interest. In Ash I, vessel 4, and Ash II, vessel 5, the tensions needed to move a continuous column upwards at the measured velocities are higher than feasible; the product lv increases rather than decreases, so that we have no case for flow under elastic expansion. On the assumption that the vessels contain gas, but that the cut is made under the water-level, however, the rising value of lv is readily explained. The length of the water column must obviously be added to that of the ink in order to obtain a true measure of the gas pressure in such cases; and it is readily deduced that by adding 28 and 20 cm. respectively to the readings of l in these two vessels lv becomes approximately constant. The gas pressure then, however, falls well below zero. These determinations fulfil none of the conditions set out above. Ash I, vessel 19, and Ash III, vessel 7, present a similar problem, although in the latter case the velocity is quite constant and in the former approximately so

except for the last determination. Here we may again conclude that the cut is made below the water-level in the vessel, but again the pressures calculated (on the basis that the pressures for each reading must lie within 3 per cent.

TABLE III

Uncertain

Specimen.	Diameter (mm.).	Velocity, <i>v</i> (cm./sec.).	Height, <i>l</i> (cm.).	<i>lv.</i>	Gas pressure in vessel (atm.).	Water tension in vessel (atm.).
<i>Ash I</i>						
vessel 4	0.136	44.0	6.4	282	0.51	78
		41.0	8.9	363	0.37	
		40.0	11.2	450	0.22	
		38.0	13.5	514	0.10	
		29.0	15.4	447	0.20	
19	0.148	42.0	6.9	292	0.58	62
		40.0	9.3	374	0.46	
		44.0	11.8	500	0.28	
		27.0	13.8	375	0.46	
<i>Ash II</i>						
vessel 4	0.242	67.0	7.0	469	0.73	49*
		43.0	10.0	430	0.77	
		31.0	13.0	403	0.78	
		20.0	16.0	320	0.83	
		13.0	19.0	247	0.87	
5	0.220	93.0	5.0	465	0.69	75
		77.0	10.0	770	0.48	
		62.0	15.0	930	0.39	
		48.0	20.0	960	0.37	
<i>Ash III</i>						
vessel 7	0.242	60.0	7.0	420	0.77	39
		(const.)				
			17.0	1020	0.44	

* This series may be explained either by assuming the vessel to contain gas, and the cut to be made some 65 cm. below the vessel termination; or by elastic expansion of a vessel filled with water, the corresponding exponential equation being

$$\text{Tension} = (0.31)e^{(-0.92)t}.$$

of each other) falls well below zero. These four observations are unique in the present investigation. Many more vessels have been investigated than are tabulated in this paper, although results obtained from them have been rejected because the shape of the vessel cross-section was too irregular to allow the application of any rigid formulae. Calculations of *lv*, however, show that none of these observations would be included with those considered in this paragraph. A possible explanation of these four results would seem to be that the vessels to which they refer lie near vessels, on the surface of the wood, containing continuous water columns (see below). Two possible conditions may exist. Either the water they contain as continuous columns is being withdrawn into these neighbouring conducting systems; or the vessels contain water and water vapour, the rising level of the water, after injection,

bringing free water into contact with the walls of unopened vessels (with continuous columns) which proceed to absorb it by elastic expansion. These would represent highly exceptional conditions for, as has already been pointed out, neighbouring vessels can usually be injected individually. In either case v will tend to decrease, whereas the value of l bears no constant relationship to the length of column moving; and the additional forces brought into play as more and more conducting systems become available may well cause v to remain at a level higher than we should expect from the injection of isolated vessels.

DISCUSSION AND CONCLUSIONS

While the conclusions which may be drawn from the foregoing observations must be only tentative, and any general statement must await more comprehensive series of observations, the present experimental results on analysis appear to be intelligible. It would appear that many of the vessels are filled with gas at reduced pressure while others contain continuous water columns: and even in these latter cases there is no evidence of very high tensions. The initial rate of injection in many of those vessels which contain liquid columns is apparently due to vessel expansion. Whether such vessels inject over distances as long as those containing air is a subject for future research, since the present calculations were made long after the trees in question ceased to be available. It is to be expected that vessels containing water under tension will cease to inject further after a comparatively short time. The possibility that the apparent movement into a partial vacuum is due simply to the breaking of a continuous water column consequent upon the insertion of the knife may be disregarded. Under these conditions the 'gas-pressure' in the vessel should be approximately zero, whereas it is, in fact, rather high.

As regards the development of the pneumatic system in vessels, and the distribution of gas-filled vessels in relation to water-filled, certain aspects are fairly clear. The present investigation included some determinations on this phase of the problem, and this work is mentioned here rather than in the body of the paper only because it is of an entirely different type. It is quite clear that the newly differentiated vessels are filled with sap up to the time when the protoplasmic contents of the vessel elements begin to disappear (Priestley, Scott, and Malins, 1935). Once the vessel becomes mature, however, its contents are presumably being withdrawn into a leaf above. We may therefore expect comparatively high tensions to be set up in such a liquid column at this time. Dixon (1914), Ursprung (1915*a*), and others have repeatedly called attention to the fact that water contained in tonometers can withstand much higher tensions than any we may expect in the tree. Their experiments were made, however, on small volumes of water, held in glass containers; and in those experiments in which a leafy shoot has been used to raise mercury above barometric height, clean glass tubes, clean water, and shock-proof

supports are essential to success. Further, Ursprung has shown that water saturated with air is unable to withstand the high tensions observed with pure water, and that the presence of dissolved gases causes considerable inconsistencies in the results (1915a).

Now the water in the tree undoubtedly contains a considerable quantity of dissolved gas and the vessel system is, moreover, by no means rigidly supported along its whole length. Further, the walls of the vessels, unlike those of the glass tubes used in laboratory experiments, are not completely smooth, particularly in the case of the protoxylem elements in which each longitudinal file of vessel elements must end. Although it may be questioned whether the nature of these projections (the bordered pits of secondary xylem and primary metaxylem and the spiral thickening of the protoxylem elements) is such as to be effective in causing the production of gas or vapour bubbles, their presence will, at least, lend no support to the continued existence of liquid columns; and in consideration of the rapid movement of the branches and the fact that the water is not gas-free, the conclusion seems inevitable that the columns will sooner or later break. The bubble of vapour thus produced would, of course, enlarge, due to the withdrawal of the now 'free' water into differentiating vessels and into such liquid columns as remain intact. At the same time air would diffuse into the bubble from the atmosphere via solution in the (wet) walls. While, therefore, we might expect continuous columns on the surface of the new wood, older vessels, farther in, may have gas under progressively higher pressures. That such gas-filled vessels may later fill again with water, by the absorption of oxygen in living wood parenchyma and the subsequent diffusion to the atmosphere of the nitrogen (under atmospheric pressure when all the oxygen is removed) seems unlikely, particularly in vessels more than 8 ft. long (about $\frac{1}{2}$ of barometric height in water).

In support of the above considerations is the well-known fact that heartwood generally contains more air than sapwood, and the following investigations made during the course of the present research tend to show a gradient of gas-pressure even in the sapwood itself. For these determinations straight branches were chosen of *Acer saccharum* and *A. saccharinum* (whose vessels are short—some 2 to 3 ft.), about 4 ft. long, the bark was removed and the twig injected at the middle point with a boiled solution of Sudan III in oil. This particular injecting fluid was chosen since it was readily visible, did not stain the walls, and could not cause blocking of the vessels by infiltration during the time which elapsed between injection and observation. Injections were made both before and after the removal of the branch from the tree. Each twig individually was then placed in a glass tube which was slowly evacuated, and both the oil meniscus and the cut were observed under dissecting microscopes. Immediately after the evacuation of the tube was commenced (certainly before the pressure in it had fallen to 65 cm. of mercury) and before any movement of the oil menisci could be detected, oil

began to collect in the incision. This oil could have come only from the vessels deep in the wood which were not available for observations. At lower pressures the deeper, visible menisci began to be withdrawn and these menisci moved forward again on letting air into the apparatus. The oil in several of the surface vessels, on the other hand, was not withdrawn appreciably under pressures as low as 2 cm. of mercury. These observations can only mean that many of the vessels contain gas under reduced pressure, and it was hoped from this kind of experiment to obtain independent measures of the gas-pressures as a check on the results presented above. Unfortunately no injecting liquid was discovered sufficiently free from 'sticking' to give constant results. The development of this technique is a subject for further research, yet at the same time these preliminary observations are not without significance. They allow the tentative conclusion to be drawn that the vessels containing gas are located deeper in the wood than are those containing liquid columns, just as theoretical considerations have suggested.

The question of the path of water movement in the tree would thus appear to require reconsideration. Vessels containing gas, even under very much reduced pressures, are almost certainly incapable of conveying water to the foliage and probably serve merely as reservoirs. In the first place, measurements by many investigators (Clum, 1926; Curtis, 1936; Smith, 1909) have shown quite clearly that leaf temperatures may be considerably higher than the temperature of the surrounding air, and therefore presumably higher than that of the wood of the branches and trunk. In such liquid-vapour systems as we have here, therefore, we would expect distillation downward rather than upward (the increase in vapour pressure of the leaf mesophyll due to the increased temperature more than offsets the decrease due to the concentration of the vacuolar sap).¹ In the second place, the pressure difference of water vapour between the two ends of the system could not be greater than a few centimetres of mercury, and it may be shown that such pressures are totally inadequate.² It would seem that no definite conclusion can be reached concerning the path of water movement until we can ascertain the rate of water loss by the tree in relation to the anatomical structure of the trunk wood. Such determinations are unfortunately lacking in the literature, though experiments have been commenced on these lines at Leeds from which results are hoped for in the near future.

¹ e.g. the vapour pressure of water at 21° C. is 18.1 mm. At the same temperature, a 1.75 M solution of K₂CO₃ has a vapour pressure of 16.8 mm., a decrease of 1.3 mm. Pure water at 22° C. has a vapour pressure of 19.66, an increase of 1.56 mm. Thus a rise of only one degree in temperature more than balances the effect of a concentration of solutes higher than any we should expect in the mesophyll.

² Rough estimation from transpiration experiments now in progress shows that the velocity of the rise of water in the experimental trees is of the order of 1/360 cm. per sec. The corresponding velocity of water vapour is about 160 cm. per sec. In a vessel 100 cm. long (the height of the trees) and 0.05 cm. radius the pressure difference required would be approximately one atmosphere. This pressure difference is, of course, impossible to conceive.

SUMMARY

When the wood of a ring-porous tree is cut under coloured liquid, during spring, the new vessels may be seen to inject very rapidly over considerable distances. Such injection may be explained either by continuous liquid columns under moderately high tensions or by the presence in the vessels of gas under reduced pressure. In the present paper it is shown that calculations made from the rate of injection (measured by a motion-picture camera) are of critical importance in determining which of these two explanations holds in specific cases. It is shown that in *Fraxinus americana* many of the vessels contain gas under pressures varying from 0.4 to 0.9 atmosphere; while in those vessels which contain liquid columns the initial expansion is often due solely to elastic expansion.

On the basis of observations made on specimens of *Acer saccharum* and *A. saccharinum*, it is suggested that the vessels which contain gas are located deeper in the wood than are those with continuous liquid columns. In view of the fact that gas-filled vessels are almost certainly functionless as conductors of water it is suggested that the whole question of the ascent of sap needs reconsideration.

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Protein Breakdown during Germination of *Lathyrus odoratus*

BY

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With one Figure in the Text

IN their paper on the respiration during germination of *Lathyrus odoratus*, L., var. 'What Joy', Stiles and Leach showed that the intensity of respiration as indicated by CO₂ evolution rises to a maximum and then falls to a more or less constant level. In the case of seeds from which the testas are removed before germination, this maximum is reached fairly regularly within the first thirty hours of the experimental period (Stiles and Leach, 1932).

Although carbohydrates are the chief storage materials in the seeds of *Lathyrus odoratus*, proteins are also an important storage food material. It was found by analysis that the total nitrogen content of these seeds (without testas) is about 5.2 per cent. Of this about 4.66 per cent. is protein nitrogen, and if this value is multiplied by the conventional factor 6.25, a value of 29.13 per cent. is obtained for the protein content. These percentages are expressed in terms of fresh (undried) tissue taken as 100 per cent. If these values are expressed in terms of dry weight, they are somewhat more impressive and give a more accurate idea of the proportion of proteins in relation to other classes of food materials. On a dry weight basis the total nitrogen is 6.00 per cent.; protein nitrogen 5.37 per cent.; and multiplying by 6.25 we get 33.56 per cent. as a figure for protein.

With such a considerable proportion of protein present the question naturally arises as to the extent to which the curve of CO₂ evolution represents the energy released during the early stages of germination. If there is a rapid initial breakdown of protein there will be more energy available than is represented by the CO₂ curves, although an amount of energy equivalent to that released in protein breakdown may later be absorbed in the resynthesis of protein at the growing points of the young plant. Again, the time of maximum protein breakdown might not be coincident with the maximum of CO₂ evolution, which would mean that the form of the respiratory curve as indicated by CO₂ evolution would not be an exact representation of the sequence of energy release. Lastly, it might be that the curve of protein breakdown does not show a rise to a maximum, the rate of breakdown being fairly even.

With a view to throwing light on this problem the following investigation was undertaken.

METHODS

The estimation of nitrate nitrogen in protein-free extract of ungerminated seeds.

A preliminary analysis of a protein-free water extract of *Lathyrus odoratus* seeds (including testas) was carried out by Mr. R. G. Westall at the Imperial College by the courtesy of Professor A. C. Chibnall. This analysis showed the presence of nitrate nitrogen to the extent of 11.6 per cent. of the total non-protein nitrogen or 0.052 per cent. of the oven-dried tissue.

It was decided to work throughout with seeds from which the testas were first removed (see p. 25) and as it was possible that part or the whole of the nitrate might be stored in the testa, a water extract of testas was prepared and tested for nitrates: (i) qualitatively with diphenylamine sulphate and concentrated sulphuric acid; (ii) quantitatively using Chibnall's micro-modification of Vickery's reduced iron method. In both cases a negative result was obtained indicating the absence of nitrates from the testa.

The presence of nitrates involved the adoption of suitable modifications of both macro- and micro-Kjeldahl methods.

Total nitrogen. Macro-Kjeldahl estimations modified for the presence of nitrates.

Preparation of protein-free extract. Barnstein's modification of the Stutzer process¹ was used for the precipitation of protein. This mode of separating out the proteins depends upon the fact that the proteins form insoluble compounds with copper hydroxide while the water soluble nitrogenous compounds of plant extracts form soluble salts.²

The fluid containing the protein precipitate was filtered on a Buchner funnel; the precipitate was then collected and boiled again with distilled water and a second bulk of filtrate obtained. The filtrate was concentrated to small bulk at a temperature of about 45° C. at reduced pressure and finally filtered through a 'Seitz-Werke' filter.

Thymol was used as a preservative.

Total non-protein nitrogen. Total non-protein nitrogen was determined by the micro-Kjeldahl method (modified for the presence of nitrates)³ devised in Professor Chibnall's laboratory.

Pregl's modification of Parnas and Wagner's micro-Kjeldahl apparatus was used, except that the double-walled distillation flask with a vacuum between the two walls was replaced by a flask which could be easily detached from the rest of the apparatus. This was necessary for cleaning purposes since iron

¹ See Allen's Commercial Organic Analysis, vol. viii, p. 662, 5th edition.

² Kostychev and Brilliant have shown that at low temperatures amino-acids and sugars may combine to give complexes which can be precipitated by copper sulphate (see Robinson, 1929). In this way the nitrogen precipitated as protein nitrogen becomes slightly increased. In regard to the present investigation it may be noted that the protein was precipitated from a hot 'solution', and that at best, the results obtained would be affected only in the sense of lessening somewhat the degree of protein breakdown.

³ A micro-modification of Vickery's reduced iron method (Pucher, Leavenworth, and Vickery, 1930). Kahlbaum reduced iron was found to give satisfactory results at the Imperial College.

was used for the reduction of the nitrates present. A full description of Pregl's modification of the Parnas-Wagner apparatus will be found in 'Pregl's Quantitative Organic Micro-analysis' (Roth, 1937).

Alizarin red (a 1 per cent. solution) was used as indicator.

Protein nitrogen. This value was obtained by deducting the total non-protein nitrogen from the total nitrogen.

Amino-nitrogen. Total amino-nitrogen was estimated by means of a micro van Slyke apparatus (Chibnall and Westall, 1932) and also by the Willstätter titration method (Willstätter and Waldschmidt-Leitz (1921)).

The amino-nitrogen values quoted in this paper were determined by Mr. R. G. Westall at the Imperial College by the courtesy of Professor A. C. Chibnall.

For this purpose protein-free water extracts were prepared.

Germination. After removing the testas, the seeds were placed on moist filter paper in half Petri-dishes and allowed to germinate in the dark in an electric oven at 25° C. The filter paper was frequently moistened, and so the addition of excess water was avoided.

Preparation of seedling material for analysis. At the end of a period of germination the seedlings were removed from the incubator, pressed gently between sheets of filter paper, and placed on watch-glasses in an electric oven at 80° C. After a few hours the seedlings were taken out of the oven and ground to a fine powder which was then returned to the oven and dried to constant weight.

The total loss in weight, total nitrogen, total non-protein nitrogen, and protein nitrogen were calculated in terms of the 'fresh' weight of the tissue.

Size of seed sample and reasons for the removal of the testa. Samples of 50 to 120 seeds were used for each experiment.

There is considerable variation in the amount of germination within a given period shown by individuals of a batch of seeds. Thus ideally it were best if the rate of protein breakdown could be followed for a single seedling. The principle of studying the behaviour of a single seedling is practicable in the case of CO₂ evolution if use is made of the katharometer (Stiles and Leach, 1931), but from the nature of the case this is not possible where chemical analyses are concerned, since it is necessary to kill a seedling to make an analysis. This of itself limits the application to biochemical and physiological investigation of such delicate methods as those of Pregl¹ since considerable individual variation occurs in the amount of any class of organic substance present in the tissues of organisms. Where chemical analyses have to be made on tissue material it is an advantage to work with fairly large samples even when a pure strain is used for the investigation.

The results of chemical analysis with fair-sized samples should agree fairly closely in accordance with the principle of variation around a mean. In considering the germination of seeds, however, other factors have to be taken

¹ In addition to this, the Pregl methods are essentially a system of elementary analysis.

into consideration. These more particularly concern the testa, which may vary in thickness and certainly varies in its degree of looseness or tightness. Again, the testas of some seeds show a slight break in the region of the radicle, some distance above the tip. From such facts as these it is to be expected that seeds with testas left intact will show greater variation in amount of germination within a given time than seeds from which the testas have first been removed. This view is borne out by the comparisons made by Stiles and Leach of CO_2 output during the early stages of germination in *Lathyrus odoratus* seeds with and without testas, for the sequence of CO_2 evolution during the first few days of germination is much more regular in seeds from which the testas have first been removed (Stiles and Leach, 1932).

A second reason for removing the testa is the nature of the testa itself, which is horny and difficult to pound into a powder, and thus the inclusion of testa in ground tissue would lead to greater heterogeneity and so would increase sampling errors.

The seeds of *Lathyrus odoratus* are not easily attacked by fungi and bacteria even after the removal of the resistant testa. Those few seedlings which were attacked by parasitic organisms were dried separately and the dry weight added to that of the rest of the batch, but they were not included in the tissue used for analysis.

EXPERIMENTAL

Loss of respired material. Below are given figures for the loss in weight during the stated intervals. In each case the loss in weight is corrected for the water content of ungerminated seeds, and thus the value given represent loss of respired material.¹

0-2 days. 5.18%	2-4 days. 2.83%	4-6 days. 1.43%	6-8 days. 3.59%
8-10 days. 2.39%	0-1 day. 3.19%	1-2 days. 1.99%	

The maximum loss in weight takes place during the first forty-eight hours, and particularly during the first twenty-four hours. During this same period, as would be expected, Stiles and Leach found the maximum output of CO_2 in seeds divested of testas.

Protein breakdown during a ten-day germination period. The results are summarized in Table I.

The data presented indicate that during the first two days of germination, when CO_2 evolution and loss of respired material are at their maximum, there is but little protein breakdown. After the first two days the rate of protein breakdown increases very markedly until the sixth day of germination.

¹ The percentage values for a given time interval were obtained by subtracting from the total loss in weight the total loss in weight for the preceding time interval. The loss in weight for the first twenty-four hours was obtained by subtracting the average water content of ungerminated seeds from the total loss in weight.

From the sixth to the tenth day the rate of protein breakdown remains fairly constant, but is at a lower level than during the second to sixth day, but at a decidedly higher level than during the first to second day of germination (Fig. 1).¹

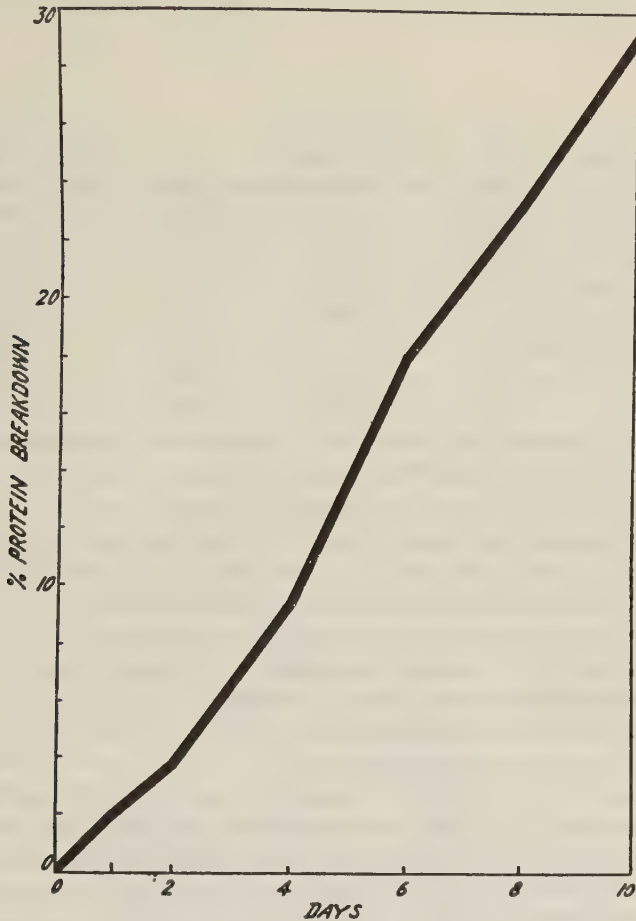


FIG. 1. Graph showing extent of protein breakdown at different time intervals during germination of *Lathyrus odoratus*.

Protein nitrogen of ungerminated seeds (4.66 per cent.) taken as 100. Value for protein nitrogen obtained in every case by subtracting total non-protein nitrogen from total nitrogen content of ungerminated seeds (Table I, fourth column).

It should be noticed that after two days' germination the total nitrogen falls to the level of 4.9 per cent., showing a tendency to decrease in value as the

¹ The curve obtained from the data given in the last column of Table I differs from Fig. 1 in that the rate of protein breakdown is somewhat greater between the second and fourth day as compared with the fourth to sixth day in Fig. 1. In both cases the period of greatest protein breakdown occurs between the second and sixth day of germination.

period of germination is extended. Consequently there are two possible values for the protein nitrogen content of seeds after ten days' germination, viz. non-protein nitrogen content of seeds subtracted from the value obtained for total nitrogen in ungerminated seed material, and, secondly, the non-protein nitrogen subtracted from the value obtained by direct determination

TABLE I

Length of germination period, days.	Total loss in weight, per cent.	Total non-protein nitrogen, per cent.	Protein nitrogen (5.2% nitrogen minus non-protein nitrogen), per cent.	Total nitrogen, per cent.	Protein nitrogen (total nitrogen determined for each experiment minus non-protein nitrogen), per cent.
0	12.59(4)*	0.545(2)	4.66(2)	5.21(2)	4.66
1	15.78(5)	0.626(2)	4.57	5.19(3)	4.56
2	17.76	0.698	4.50	5.3	4.60
4	20.59	0.970	4.23	4.92	3.95
6	22.02(2)	1.370	3.83	4.96	3.59
8	25.61	1.612	3.59	4.94	3.33
10	28.00	1.877	3.32	4.875	3.00

* The figures in brackets indicate the number of estimations (as distinct from 'duplicate determinations') from which the average value is obtained.

of total nitrogen in seeds after ten days' germination. The values are 3.33 per cent. and 3.00 per cent. respectively. Taking the protein value of ungerminated seeds (29.13 per cent.) as 100, we get 28.7 and 35.67 as the corresponding values for the percentage protein breakdown.

Nature of the slight decrease in total nitrogen content. The decrease in total nitrogen recorded after two days' germination is probably due to the exosmosis of water-soluble nitrogenous compounds. This seems especially probable as the period of greatest rate of protein breakdown and that of decrease in total estimated nitrogen coincide.

In this connexion attention may be called to the work of Davidson (1923) regarding the constancy of the nitrogen content of germinating wheat and cow pea seedlings. These investigations showed that when seeds were germinated and subsequently digested in the same Kjeldahl flask, no significant fluctuations were obtained in total nitrogen content during germination. This, of course, is what would be expected from the *corpus* of investigation and theory concerning the nitrogen metabolism of the higher plants. Still, a loss of nitrogen has been claimed by some investigators.

Increase in amino-nitrogen during the first three days of germination. The following values were obtained for amino-nitrogen by the micro Van Slyke method (Chibnall and Westall, 1932).

	Per cent. of total non-protein nitrogen.	Per cent. amino-nitrogen on a dry weight basis.
Ungerminated seeds	26.2	0.121
Seeds germinated three days	37.2	0.329

Estimations were also carried out, using the Willstätter titration method for amino-nitrogen, and the following values obtained:

	Per cent. of total non-protein nitrogen.
Ungerminated seeds	23.5
Seeds germinated three days	33.0

DISCUSSION

A germinating seedling presents a dual system. The food storage tissues are centres of breakdown of more complex to less complex and soluble substances and the growing regions are centres where there is active building-up of cell structures and protoplasm. The chief group of chemical substances involved will vary with the species. In most cases the chief storage products are fats (Stiles and Leach, 1932*a*), in others polysaccharides (usually starch), and in the smallest group proteins at least form a considerable percentage of the stored material. A great deal of the breakdown products in the case of oils and carbohydrates become dissipated as carbon dioxide and water in the process of respiration. In this way, as also by the breakdown of more complex to simpler compounds, energy is liberated, some of which is utilized by the growing plant while the remainder is radiated as heat. Some of the energy liberated in the storage centres by the breakdown of more complex substances will have to be replaced in resynthesis of these compounds in the growing regions. This applies more particularly to proteins.

During germination, protein is broken down in the cotyledons into soluble nitrogen compounds which are transferred to the growing plumule and radicle where resynthesis of protein is taking place. If, however, the rate of protein breakdown is markedly in excess of the rate of protein resynthesis, a surplus of energy will become available to the young seedling.

It has been shown that an increase in the amino-acid content of leaves results in an increased liberation of carbon dioxide (Spoehr and McGee, 1923). Thus in the case of germinating seedlings which are not suffering from carbohydrate starvation, it would seem that an excess of protein breakdown over protein resynthesis might result in energy derived from the stimulating effects of accumulating amino-acids on carbohydrate respiration in addition to the energy from excess protein breakdown over protein resynthesis. The maximum CO₂ evolution of germinating *Lathyrus odoratus* seeds deprived of their testas was, however, usually recorded by Stiles and Leach within the first thirty hours; while this investigation has shown that there is but little protein breakdown during this period. At the end of three days germination, the amino-nitrogen had increased from 0.121 per cent. to 0.329 per cent., expressed on a basis of dry weight of tissue. These data indicate that amino-acids were accumulating when the rate of CO₂ output had already passed the maximum and had reached a more or less constant level. The

figures for total loss in weight show the same essential sequence as the CO_2 evolution curves. This would seem to indicate that the energy liberated during the earlier period of germination may be far in excess of that needful for the metabolic activity of the seedling, or else that the seedling makes use of the increasing energy which becomes available through the widening ratio of energy liberated by protein breakdown to energy utilized in the resynthesis of protein.

The possibility of protein respiration as distinct from protein breakdown has to be borne in mind. In order to throw further light on this question it would be necessary to carry out a series of analyses on the changes in distribution of the soluble nitrogen fractions during germination. A marked fall in amino-acid nitrogen might be significant, also an accumulation of amide nitrogen or ammonia nitrogen, as indicating the respiratory decomposition of organic acids ultimately derived from proteins.

SUMMARY

1. Analyses were carried out on seeds divested of their testas and with samples of 50 to 120 seeds.

2. Protein was precipitated by the Barnstein modification of the Stutzer process and protein nitrogen estimated by deducting the total non-protein nitrogen determined by the micro-Kjeldahl method from the total nitrogen determined by macro-Kjeldahl estimations. Both macro- and micro-Kjeldahl estimations were modified for the presence of nitrate which occurs in the embryo but not in the testa.

3. The total nitrogen of ungerminated seeds of *Lathyrus odoratus*, L., var. 'What Joy', is 5.2 per cent. on a fresh weight basis. About 4.7 per cent. of this is protein nitrogen. Multiplying the protein nitrogen by the conventional factor, 6.25, a value 29.13 per cent. is obtained for protein (33.6 per cent. on a dry weight basis). Thus about one-third of the total organic matter of ungerminated seeds of *Lathyrus odoratus* consists of protein.

4. During the entire experimental period of ten days' germination about a third of the protein undergoes decomposition.

5. During the first two days of germination, when the carbon dioxide output and loss of respirable material are greatest, there is but little protein breakdown.

6. The greatest intensity of protein breakdown occurs from the second to the sixth day of germination.

7. From the sixth to the tenth day the rate of protein breakdown is more or less uniform and proceeds at a lower level than from the second to sixth day, but at a higher level than during the first two days.

8. Amino-nitrogen increases from 0.121 per cent. to 0.329 per cent. during the first three days of germination.

This work was carried out in the Botany Department, Birmingham University. I wish to thank Professor A. C. Chibnall for his valuable advice and help and for his courtesy in allowing the writer to learn the semi-micro methods of protein analyses in his laboratory.

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Studies on the Nitrogen Metabolism of Plants

I. The Relation between the Content of Proteins, Amino-Acids, and Water in the Leaves

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With seven Figures in the Text

INTRODUCTION

OUR knowledge of certain aspects of plant metabolism has been considerably advanced by studies of the dynamics of the processes concerned. This has not been the case for nitrogen metabolism, doubtless mainly because of the difficulties of measuring momentary rates; here the approach has been made by the study of the relations among the amounts present in the tissues of various substances that are affected by the metabolic sequences. Much quantitative information, however, is still required about the factors determining these relations, in order to throw further light on the nature of the physiological processes concerned. The present work is devoted to the investigation of such determining factors.

THE REACTIONS INVOLVED IN NITROGEN METABOLISM

As a basis for this work a schema is presented which seems the best representation obtainable from existing knowledge of the sequences of reactions involved in nitrogen metabolism. References to the older literature are omitted, as they may be found in works such as those of Onslow (1931) and Robinson (1929). The schema is confined to the system represented by the leaves of the plant, and the case will be considered where the plant is supplied with nitrogen in the form of ammonium compounds. Consideration is omitted of the case of the plant supplied with NO_3^- ions and of any possible formation of these ions in metabolism, but the schema would cover the case

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of the plant supplied with amino-acids or amides. The following processes are then recognized: (1) The arrival of ammonia-nitrogen in the leaves. (2) The formation of glycolysis products in respiration, and their disappearance in oxidation to carbon dioxide, in conversion to organic acids and in other ways. (3) The synthesis of amides (mainly asparagine and glutamine) from ammonia and organic acids, derived as in (1) and (2) (Mothes, 1933*a*; Virtanen and Tarnanen, 1932). Amides may also be broken down to re-form ammonia and carbohydrate residues. (4) The combination of compounds derived from glycolysis products with ammonia nitrogen to form amino-acids, and their de-amination to re-form ammonia nitrogen and organic acids. (5) The synthesis of proteins. It is not yet known whether amino-acids condense to form proteins, or whether they are produced only on protein hydrolysis,¹ the upgrade process following another path. Grassmann (1932) states that the hypothesis that proteins are built up *en bloc* from separately synthesized units more complex than amino-acids (cf. Onslow, 1931) is now regarded as less probable. On the other hand, Alcock (1936) suggests that a simple unit is first formed, which polymerizes to form a basal substance from which the various proteins are subsequently differentiated. (6) The combination of ammonia nitrogen with various substances to form other nitrogen compounds grouped together as residual nitrogen;² these can probably be broken down again to the compounds from which they are derived. (7) The translocation of synthesized compounds away from the leaf. All the crystalloidal compounds of nitrogen are probably capable of being translocated.

We thus visualize a network of reactions. It is possible that, where a substance is synthesized and hydrolysed, the two processes may each proceed along alternative paths, but the natural tendency to proceed along one path in a certain direction may be opposed by other metabolic activities. It is in fact possible that, whether one or more intermediate paths exist, the reactants and products in certain reactions in this schema never reach equilibrium with one another but are maintained at non-equilibrium concentrations by the expenditure of energy by the living system.

EXPERIMENTAL METHOD

We may expect many factors to influence the rates of processes in nitrogen metabolism, and consequently the amounts of substances present in the leaf at any time. The ideal experimental method would be the alteration of these factors singly; in practice this is difficult since alteration in one factor tends to alteration in others. The method actually adopted was to submit plants, placed under constant conditions of light, temperature, and water supply, to treatments designed to cause variations in the factors whose effect it was

¹ Chibnall (1922) believes that amino-acids are built up into proteins but that degradation takes place directly into residual nitrogen: this does not seem an inevitable conclusion from his data.

² In practice diamino-groups of amino-acids are included in the residual nitrogen.

desired to investigate. With constancy of the environment there was a greater probability that a steady state might be approached in the leaves than with an environment fluctuating in a random manner. Attainment of a constant steady state was not, however, expected: the fact that the external solution drifted in composition as its components entered the plant would alone have been sufficient to prevent this. But it was possible that conditions may have been such as would facilitate an approach to a *drifting steady state*. In such a state the changes in the factors defining the state of the system are so slow that they result in a succession of states, each of which is inappreciably removed from a steady one.¹ The attainment of a drifting steady state is thus dependent upon the drift in the factors that determine the steady state being slow in comparison with the rate of approach thereto. An approach to this state was desirable since the effect of factors determining the amounts of substances taking part in metabolism might thereby be more clearly revealed.

At intervals a certain number of plants were examined with respect to the content of various nitrogen compounds in their leaves, and also with respect to other attributes that were regarded as probable factors determining these contents. Three experiments that have been carried out will be described and the results examined in detail.

DESCRIPTION OF EXPERIMENTS

Material

For each experiment, plants of a pure line grass, *Phalaris tuberosa* L. or *Lolium multiflorum* Lam., were grown in jars in the glasshouse; four were grown in each jar, which contained 3.75 kg. water-washed sand maintained at 60 per cent. of its saturation capacity by addition of distilled water. Six days after sowing, the following nutrients were applied in solution:

KCl	.	.	.	0.3 gm. per pot
(NH ₄) ₂ SO ₄	.	.	.	0.6 „ „
MgSO ₄ ·7H ₂ O	.	.	.	0.3 „ „
Ca(H ₂ PO ₄) ₂	.	.	.	0.6 „ „
FeCl ₃	.	.	.	0.015 „ „

At intervals of about three weeks, two subsequent applications were made, each of the following:

(NH ₄) ₂ SO ₄	.	.	.	0.3 gm. per pot
NaOH	.	.	.	0.06 „ „

Sodium hydroxide was added to counteract the acidity due to rapid uptake of the ammonium radicle; the amounts were chosen in the light of observations on the pH of the nutrient solutions in water.

¹ This concept is that of the 'moving equilibrium' of Lotka (1925).

Differential Treatment and Sojourn under Constant Conditions

When the plants were ready for the experiment, a number of pots were placed in two cabinets under constant conditions of light and temperature, and with a steady stream of air circulating. The light intensity was approximately 800 metre-candles, as determined by a Holophane lumeter, and the temperature 24° C. During their sojourn in the cabinets the pots were brought up to their correct water content daily.

Twenty-four hours after the pots were placed in the cabinets, differential treatments were applied. The treatments consisted of either the application of doses of nitrogen compounds, or else, in one experiment, the reduction of the water content of certain pots to a lower level. Certain pots were kept untreated. The treated and untreated pots were all distributed in a random manner. After a further period of twenty-four hours, and also on the ensuing two or three days, four pots per treatment (in Experiment III, six pots) were removed in a random manner in order that the plants might be investigated. The removal always followed the same order of treatments and was spread over eight to twelve hours on each day. This removal of pots tended to cause the atmospheric humidity in the cabinets to fall; the temperature, however, remained unchanged, and shading effects were only slightly altered.

Investigation of the Plants

In Experiments I and II three plants from each pot were used for analysis. The remaining plants provided material for respiration determination. In experiment III the plants from the extra two pots were all used for analysis. The leaves were rapidly severed at the ligule as each pot was removed; only two or three leaves at the base were dead or senescent, and these were rejected; the rest were weighed in groups corresponding to the pots.

The leaves for analysis were bulked and cut into lengths of $\frac{1}{4}$ to $\frac{1}{2}$ in. Two 10-gm. samples were removed for estimation of nitrogen compounds; two further samples were weighed out and dried at 85° C. to constant weight for water-content determinations; and from a final sample the sap was expressed hydraulically under a pressure of 74 atmospheres, and its pH immediately measured with a quinhydrone micro-electrode.

Immediately after cutting, the material for respiration measurement was placed in chambers and a stream of carbon-dioxide-free air drawn through at a rate of approximately eleven litres per hour. Carbon dioxide was absorbed in Reiset towers, and measurements were made at 24° C. at intervals of $1\frac{1}{2}$ hours, following a stabilization period of 45 to 60 minutes. When these measurements were completed, the dry weight of the material was determined.

The samples for analysis were finely ground with purified sand and 20 ml. 10 per cent. sodium tungstate mixed with 20 ml. $\frac{2}{3}$ N sulphuric acid. The precipitate was filtered and washed, and its nitrogen content, determined by the macro-Kjeldahl method, gave the 'protein nitrogen' fraction. It must be

understood that this fraction, although so termed, may have included other forms of insoluble nitrogen. The filtrate was removed before the precipitate was washed and a 25-ml. portion was distilled with lime-water under reduced pressure at 40° C. The ammonia released was trapped in sulphuric acid and estimated by Nesslerization to obtain the ammonia-nitrogen fraction. The residue was subsequently hydrolysed and amide-nitrogen similarly determined. A 4-ml. portion of the filtrate was used for estimating amino-nitrogen by the van Slyke method, and a 5-ml. portion was set aside for total-soluble nitrogen determination by the micro-Kjeldahl method. A final portion of 1 or 2 ml. was used for reducing-sugar determination by Benedict's modification of the Folin-Wu method.

The procedure of the harvest days was carried out without delays by a team of workers. Care was taken to harvest, prepare, and analyse the material as rapidly as possible to avoid the onset of changes in composition.

Expression of Results

Using the mean dry weights determined from the cut samples and the respiration material, the analytical and respiration data were placed on a dry-weight basis. The amide-nitrogen values were doubled in the usual manner to give total nitrogen in the amides, and the residual amino-nitrogen was calculated by deducting the original amide-nitrogen values from the total amino-nitrogen values.

As the respiration rate declined with time, an extrapolation was performed for each sample of material to obtain an estimate of the value appropriate to the time at which the sample was placed in the dark in the respiration chambers.

From the dry-weight determinations on the bulked leaves and the fresh-weight values for each of the four pots used for analysis, an estimate was made of the dry weight of the leaves of each pot (excluding those of the plants used for respiration determinations).

Experiment I

Seeds of *Phalaris tuberosa* L. were sown on March 5, 1935. The pots were placed in the cabinets on June 3. Seven treatments were applied on June 4. They were as follows:

C	.	.	0	gm. (NH ₄) ₂ SO ₄ per pot
N ₁	.	.	1.0	" " "
N ₂	.	.	2.0	" " "
N ₃	.	.	4.0	" " "
N ₄	.	.	6.5	" " "
K	.	.	0	gm. (NH ₄) ₂ SO ₄ + 6.5 gm. K ₂ SO ₄ per pot
KN	.	.	1.0	" " + 5.5 " " "

Plants were removed for investigation on June 5, 6, and 7. The results are given in Tables I and IV.

Experiment II

Seeds of *Phalaris tuberosa* L. were sown on May 25, 1935. The sojourn under constant conditions extended over five days, commencing on September 14. Harvests were made each day on September 16–19. The pots for the first three of these days were placed in the cabinets on September 14, and those for the last day on September 16. The following treatments were applied twenty-four hours after the appropriate pots had been placed in the cabinet:

C	.	.	No extra nutrients
N ₁	.	.	2.0 gm. (NH ₄) ₂ SO ₄ per pot
N ₂	.	.	4.0 " " "
N ₃	.	.	7.0 " " "
A ₁	.	.	2.0 gm. asparagin per pot
A ₂	.	.	4.5 " " "
A ₃	.	.	7.9 " " "

Pots of only certain of the treatments were examined each day. In the pots of C, day 4, the water content was allowed to fall. The results are given in Tables II and V.

Experiment III

Seeds of *Lolium multiflorum* Lam. were sown on June 26, 1935. The pots for examination on the first two days were placed in the cabinets on November 19, and those for examination on the third day on November 21. Five treatments were applied twenty-four hours after the pots had been placed in the cabinets. They were as follows:

C	.	.	0 gm. (NH ₄) ₂ HPO ₄ per pot
N ₁	.	.	1.00 " " "
N ₂	.	.	3.00 " " "
N ₃	.	.	5.00 " " "
N ₄	.	.	7.25 " " "

Plants were removed for investigation on November 21, 22, and 23. The results are given in Tables III and VI.

Ammonium phosphate was used in this case instead of ammonium sulphate, as the experiment was designed for the additional purpose of studying sulphur metabolism, and it was desired that the SO₄-ion supply should remain constant.

DISCUSSION OF RESULTS

The Dry Weight Data

Some interest attaches to the examination of the dry weight data, the mean values of which, with results of analyses of variance, are presented in Tables I–III.

TABLE I
Mean Dry Weights, Experiment I

Day	Treatment.							Day mean.
	C.	N1.	N2.	N3.	N4.	K.	KN.	
1	2.90	2.32	2.82	2.66	2.44	2.65	2.93	2.67
2	3.07	3.15	2.56	2.68	2.89	2.79	2.63	2.82
3	3.04	2.94	2.65	2.87	2.19	2.90	3.00	2.80
Treat- ment means	3.00	2.80	2.68	2.74	2.51	2.78	2.85	2.76

Time, treatment, and interaction insignificant.

Standard error of mean of 4 pots = 0.22.

Coefficient of variation = 7.9.

TABLE II
Mean Dry Weights, Experiment II

Day.	Treatment						
	C.	N1.	N2.	N3.	A1.	A2.	A3.
1	3.54	3.24	3.41	3.46	3.60	3.36	2.87
	C	N1	N2	N3	A1	A2	A3
2	3.55	3.42	3.76	3.27	3.81	3.48	3.58
	C	N1	N3	A1	A2a	A2b	
3	3.77	3.96	3.89	3.33	3.27	3.57	
	Ca	Cb	Cc	N2			
4	2.22	2.52	2.80	3.06			

General mean = 3.23.

Treatment significant at 1 per cent. point.

Standard error of mean of 4 pots = 0.238.

Coefficient of variation = 7.37.

TABLE III
Mean Dry Weights, Experiment III

Day.	Treatment.					Day mean.
	C.	N1.	N2.	N3.	N4.	
1	2.63	2.65	2.65	2.30	2.38	2.52
2	2.64	2.59	2.55	2.98	2.88	2.73
3	2.63	2.55	2.91	3.31	2.75	2.83
Treat- ment means	2.63	2.59	2.70	2.86	2.67	2.69

Time, treatment, and interaction insignificant.

S.E. of means of 6 pots = 0.211.

Coefficient of variation = 7.83.

A preliminary experiment, on the lines of those described in this paper, showed a significant increase in the dry weight of the leaves during the first twenty-four hours sojourn in the cabinets; but during the next two days no further significant increase could be demonstrated. The increase during the first day may have been produced partly at the expense of reserves, drawn

from other parts of the plants,¹ that had been laid down when the plants were growing at high light intensity in the glasshouse; growth thereafter may have been dependent mainly on carbohydrates synthesized under the low light conditions of the cabinets, which would account for its apparent insignificance. In view of this result, in subsequent experiments twenty-four hours were allowed to elapse before applying treatments, as it was thought that the data might be more comparable if the basis upon which they were expressed did not significantly vary.

From Table I it can be seen that neither time nor treatment could be shown to have had a significant effect on the dry weight in Experiment I.

In Experiment II (Table II) the effect of time on the dry weight could not be determined as a whole, owing to the fact that the treatments differed to a certain extent on different days. Each treatment on each day was therefore regarded as a separate one, and only the variance due to treatment was removed from the total variance. The effect of treatment was thereby found to have been significant. Where plants of the same treatment were examined on two or more successive days, however, no significant increase with time was revealed. The only significant differences in dry-weight means are the depressions produced by the low-water treatments of day 4.

Two factors might have contributed to these depressions. Firstly, the decreasing water content would increase the concentration of solutes in the leaf cells; furthermore, decreasing water content produces starch hydrolysis and high sugar concentrations (see Table V), as has already been shown by Iljin (1930), Vasiljev (1931), and others; the rate of translocation of solutes out of the leaves would probably therefore have been increased, and this would cause a reduction in dry weight. Secondly, the results show that respiration rate was increased. The rate of carbon assimilation may also have decreased with decreasing water content: decrease in rate of apparent assimilation with decrease in water content was shown by Thoday (1910), Iljin (1923), Brilliant (1924), Mayer and Plantefol (1926), Walter (1929), and Vasiljev (1931). The balance in other treatments between photosynthesis and respiration was such as to preserve the weight of the leaves approximately constant;² if then photosynthesis was decreased and respiration increased in rate, loss in weight would occur. If photosynthesis were not taking place, the measured rate of respiration on day 4 (if it had maintained the same value for the whole four days) would have been sufficient to account for a loss in weight of the order observed.

Experiment III (Table III) shows again no significant effect of time or treatment. In plants of N₃ there is a large difference between the day 1 and day 3 means, but this difference still lies within the range of experimental error.

¹ From the work of Richardson, Trumble, and Shapter (1932) it is apparent that the leaves constitute only a comparatively small portion of the plant in *Phalaris tuberosa*.

² This does not mean that the light intensity was at the compensation point, because other parts of the plant may have increased in weight.

Although no significant increase in dry weight of the leaves could be demonstrated in these experiments, this does not mean that ontogenetic drifts did not occur. Decrease in weight of senescent leaves might have compensated for an increase in that of adolescent leaves, although leaves obviously senescent were not included in the material examined. Furthermore, differentiation, morphological and chemical, might have taken place in adolescent leaves even if they did not change in dry weight, and such differentiation might cause drifts in factors determining amounts of nitrogenous substances in the leaves.

Protein-nitrogen Content and other Variables

It is proposed in this paper to confine attention to the relation of the amounts of proteins in the leaves to various factors, and to leave the full consideration of the relationships of the soluble nitrogen compounds for a subsequent paper. It is also proposed to analyse the data firstly from the viewpoint that proteins may be synthesized by condensation of amino-acids, so that we shall look firstly for a relation between the amounts of protein and amino-acid nitrogen present. Subsequently the data will be considered from the viewpoint of the other hypothesis, that proteins are produced in other ways than from amino-acids.

Experiment I.

Time drifts. These are plotted for each treatment in Fig. 1. A general tendency is revealed in the data for C-N₄¹ for protein-nitrogen content (*P*) to follow the drift of water content (*U*), which with these treatments has a minimum on day 2. Part of the drift of *U* may be due to drift in the atmospheric humidity in the cabinet, but part is also due to treatment, since the drifts in C, K, and KN are of another form. The sap pH also drifts in the same manner, except in C, where it increases with decrease in *U*. The amino-nitrogen (*A*) curves show kinks on day 2, but the drift is on the whole positive in N₁ and N₃ and negative in C and N₄; the drift of *P* is also characterized in this way. These relations are not exhibited in the same manner in K and KN, but here the different potassium content may have been a factor determining the form of the drift.

The suggestion is that *P* may be related both to *A* and *U* and possibly also to sap pH.

Treatment effects. These are plotted for each day in Fig. 2; *U* and *A* show a marked negative correlation, except between C and N₁; there is also a marked similarity in the effects on *P* and pH.

Regression functions. The data have also been examined to see whether any common relationships hold among them as a whole. If a drifting steady state had been approached at each point of analysis, and the main factors

¹ Henceforward symbols, such as C, N₁, &c., will be used to indicate plants to which the treatment so defined had been applied.

TABLE IV
Results of Experiment I

DAY 1	Treatment.						
	C.	N1.	N2.	N3.	N4.	K.	KN.
NH ₃ -N	7	8	19	110	397	16	32
2 × amide-N	149	153	167	200	509	156	172
Res. amino-N	134	151	153	178	314	179	158
Total amino-N	209	228	237	278	569	257	244
Residual N.	188	308	172	213	309	151	174
Total sol. N	478	620	511	701	1529	502	536
Protein N × 10 ⁻¹	278	289	287	283	286	254	251
Total N × 10 ⁻¹	326	351	338	353	439	304	305
Water	697	700	690	639	567	648	610
pH	5.45	6.34	6.30	5.77	6.03	6.33	5.90
Red. sugars × 10 ⁻¹	301	409	378	365	283	313	664
Resp. rate	2.5	3.2	2.7	3.9	4.6	3.5	4.1
DAY 2							
NH ₃ -N	16	14	18	147	316	19	18
2 × amide-N	88	180	184	269	308	94	136
Res. amino-N	114	139	134	196	288	115	160
Total amino-N	158	229	226	331	442	162	228
Residual N.	120	145	170	153	38	143	138
Total sol. N	338	478	506	765	950	371	442
Protein N × 10 ⁻¹	251	271	273	264	262	257	266
Total N × 10 ⁻¹	285	319	324	341	257	294	310
Water	648	606	598	579	465	669	631
pH	5.61	5.94	6.18	5.65	5.70	5.81	5.95
Red. sugars × 10 ⁻¹	545	613	438	631	483	669	434
Resp. rate	2.1	2.9	2.6	3.8	5.0	2.5	3.3
DAY 3							
NH ₃ -N	3	12	21	131	405	13	17
2 × amide-N	64	92	122	159	519	43	212
Res. amino-N	105	212	257	323	234	144	156
Total amino-N	137	258	318	403	494	166	262
Residual N.	190	361	305	205	519	198	200
Total sol. N	362	677	705	818	1677	398	585
Protein N × 10 ⁻¹	256	305	319	292	269	238	263
Total N × 10 ⁻¹	292	373	390	384	437	278	322
Water	644	710	679	619	506	630	604
pH	5.85	6.18	6.30	5.95	5.84	5.95	5.79
Red. sugars × 10 ⁻¹	495	388	476	339	471	436	377
Resp. rate	3.3	3.6	3.8	3.9	3.6	2.9	3.0

Nitrogen fractions and reducing sugars in mg. per 100 gm. dry weight.

Water in gm. per 100 gm. dry weight.

Respiration rate in mg. CO₂ per gm. per hour.

determining this state had been taken into account, such relationships would be expected. The similarity in the drifts of certain quantities does not, of course, signify that a drifting steady state was approached, as there may have been a lag in the drift of the dependent variable. However, to seek for common relationships the partial regressions have been determined of *P* on *A*, *U*, and other variables.

The results of these determinations are summarized in Table VII. In this table is included the percentage of the variance of protein nitrogen ascribable

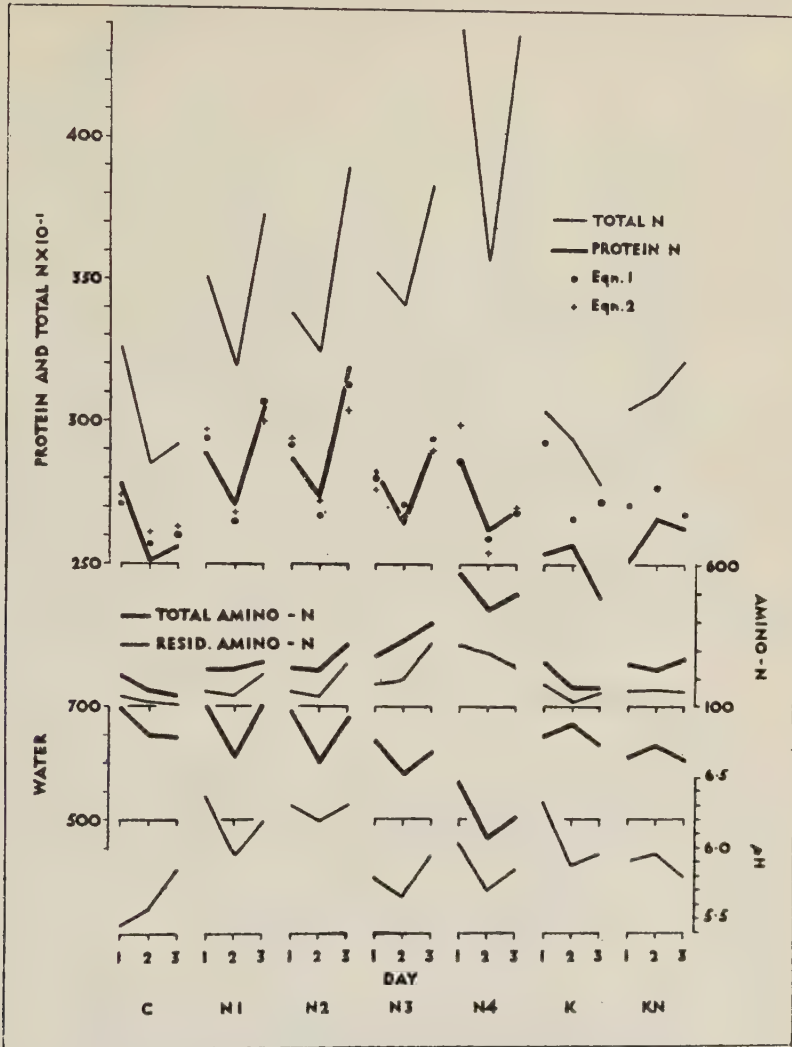


FIG. 1. Drifts with time in the contents of nitrogen compounds and water in the leaves and in the pH of the expressed sap, Experiment I. The nitrogen compound values are given in mg. per 100 gm. dry matter, and the water values in gm. per 100 gm. dry matter.

to the average effect of the independent variables; the latter quantity, denoted by V , is obtained from the equation

$$1 - \frac{V}{100} = \frac{n-1}{n-p-1} (1-R^2),$$

where n is the number of observations, p the number of independent variables, and R the multiple correlation coefficient of P with the independent variables.

Various regression functions have been fitted, and in each case the one

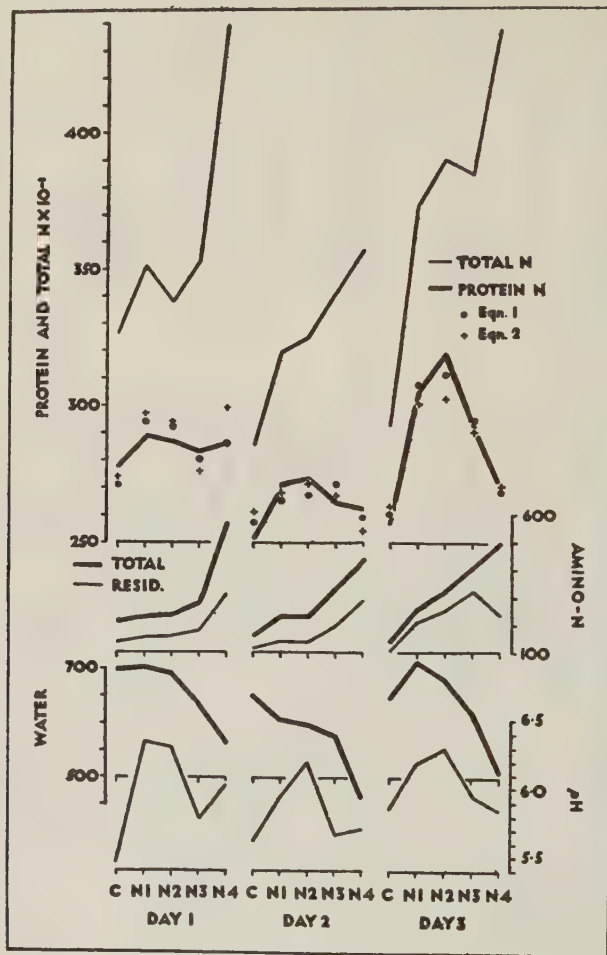


FIG. 2. Treatment effects on the contents of nitrogen compounds and water in the leaves and on the pH of the expressed leaf sap, Experiment I. The nitrogen compound values are given in mg. per 100 gm. dry matter, and the water values in gm. per 100 gm. dry matter.

yielding the highest value of V is presented; in some cases more than one is presented for comparative purposes. Separate equations are given for residual amino-nitrogen (A_R) and total amino-nitrogen (A_T), but there seems no theoretical reason why the amino-group of the amides should not take part in protein synthesis, and actually the agreement between observed and calculated values with A_R and A_T tend to be equally good. The data for K

and KN were excluded in fitting the regressions for this experiment, as it was considered that the potassium sulphate may have had a specific effect on the system. Inclusion of a term for respiration rate caused the fit to be worse.

The agreement between observed and calculated values obtained with these equations is high, as shown by the values of V . Differences between observed and calculated values are usually less than 5 per cent. of the value of P ; as the values of $P_{\text{calc.}}$ are subject to the accumulated experimental error associated with the estimation of the values of each independent variable, this degree of correspondence is clearly high. It therefore seems that, on the whole, little variation in P other than that due to experimental error is unaccounted for by the regression equations. This does not mean that pH and U are the only varying factors that have influenced the P - A relations; but other factors, if appreciable, are evidently so correlated with pH or U that the form of the function takes them into account.

Values for $P_{\text{calc.}}$ are plotted in Figs. 1 and 2 for equations (1) and (2); they do not suggest that the fit is better on any given day of the experiment, and the differences do not show any significant drift with time. The drifts with each treatment are well accounted for. It is noticeable that the most marked discrepancies between observed and calculated values occur in C and N₄, where extreme values of the independent variables are to be found. Values for K and KN in Fig. 1 were calculated from the equations obtained from the data for the plants with the other treatments. It is clear that potassium sulphate has had a specific effect.

Experiment II.

Time drifts. As all the pots were not placed in the cabinets on the same day, not all the data can be used in considering these drifts. Those plotted (Fig. 3), however, show that the drifts differ in direction from those in Experiment I; U for the most part declines with time, although the drifts and treatment effects, except in A₃, are small compared with those in the previous experiment; in fact, we here tend to have a system in which U has remained comparatively constant. Inspection of Table V shows that this holds also for pH of sap, so that we may here see the relation to A more clearly revealed. Actually the drifts in P and A follow the same direction.

Treatment effects. Fig. 4 shows that there is no marked negative correlation between U and A in this experiment. On the first three days U tends to remain unaffected by treatment, and the P drift resembles that of A ; on the fourth day the treatment was designed deliberately to alter U , and low values of U are associated with low values of A , a state of affairs not obtained in the previous experiment. Fig. 5 shows the effect of the asparagin treatments on each day: an appreciable effect occurs only on day 2 in A₃, and is associated with decrease in amount of protein; otherwise the protein curve follows the A curves.

TABLE V. *Results of Experiment II*

	Treatment.						
	C.	N1.	N2.	N3.	A2.	A3.	
DAY 1							
NH ₃ -N	9	15	17	55	23	12	
2 × amide N	—	29	82	183	147	198	
Res. amino-N	—	115	98	98	101	128	
Total amino-N	67	130	139	189	175	227	
Residue N	—	87	100	—	123	164	
Total sol. N	150	246	297	357	394	502	
Protein N × 10 ⁻¹	186	215	210	217	212	228	
Total N × 10 ⁻¹	201	240	240	253	251	278	
Water	406	422	437	425	414	428	
pH	5.89	5.85	5.95	5.69	5.97	6.37	
Red. sugars × 10 ⁻¹	295	407	256	281	301	383	
Resp. rate	1.6	3.2	4.1	6.7	3.5	4.4	
DAY 2	C.	N1.	N2.	N3.	A1.	A2.	A3.
NH ₃ -N	3	10	17	101	19	17	22
2 × amide N	32	124	129	193	124	127	172
Res. amino-N	46	103	94	144	104	123	121
Total amino-N	62	165	159	241	166	187	207
Residual N	97	174	152	171	117	146	194
Total sol. N	178	411	392	609	364	413	509
Protein N × 10 ⁻¹	184	222	228	228	228	211	184
Total N × 10 ⁻¹	202	263	267	289	264	252	235
Water	416	419	410	395	429	390	294
pH	5.81	5.80	5.96	6.05	6.87	6.02	6.33
Red. sugars × 10 ⁻¹	265	314	332	364	343	420	533
Resp. rate	3.0	3.2	3.8	4.5	3.2	3.4	3.9
DAY 3	C.	N1.	N3.	A1.	A2a.	A2b.	
NH ₃ -N	12	15	49	16	10	20	
2 × amide N	20	138	199	130	192	136	
Res. amino-N	49	124	173	125	121	115	
Total amino-N	59	193	272	190	217	183	
Residue N	84	195	143	209	150	91	
Total sol. N	165	472	564	480	474	390	
Protein N × 10 ⁻¹	179	239	224	236	225	219	
Total N × 10 ⁻¹	196	286	280	284	272	258	
Water	376	391	371	427	419	406	
pH	5.67	5.78	6.04	5.89	5.91	5.90	
Red. sugars × 10 ⁻¹	273	368	354	270	407	396	
Resp. rate	2.3	3.3	3.3	3.5	3.4	3.5	
DAY 4	Ca.	Cb.	Cc.	N2.			
NH ₃ -N	14	15	17	24			
2 × amide N	48	56	68	228			
Res. amino-N	89	63	57	162			
Total amino-N	113	91	91	276			
Residual N	173	234	217	271			
Total sol. N	312	354	342	628			
Protein N × 10 ⁻¹	158	165	163	256			
Total N × 10 ⁻¹	189	200	197	319			
Water	214	245	282	476			
pH	6.13	6.00	5.87	5.97			
Red. sugars × 10 ⁻¹	788	937	1160	791			
Resp. rate	4.6	5.0	4.4	3.9			

Nitrogen fractions and reducing sugars in mg. per 100 gm. dry weight. Water in gm. per 100 gm. dry weight. Respiration rate in mg. CO₂ per gm. per hour.

Regression functions. The data for all four days were combined to determine regression functions, which are again given in Table VII. Partial regression coefficients for respiration rate and pH were insignificant and their inclusion did not improve the fit. It was considered possible that the values for treat-

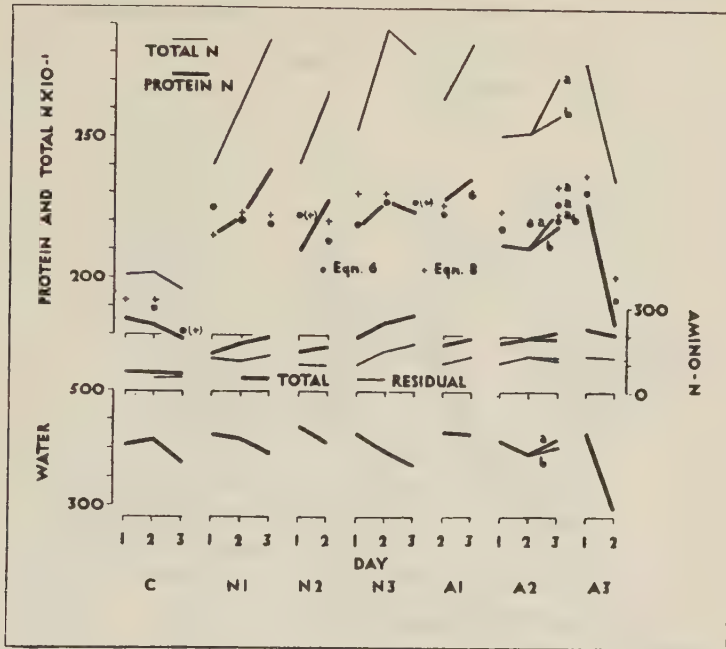


FIG. 3. Drifts with time in the contents of nitrogen compounds and water in the leaves, Experiment II. The nitrogen compound values are given in mg. per 100 gm. dry matter, and the water values in gm. per 100 gm. dry matter.

ments Ca, Cb, and Cc (day 4) may have been affected by injury resulting from the very low water contents, or have been distorted by the loss in dry weight (see Table II). Equation (3) was therefore fitted omitting these values; from the equation, protein values were then calculated for the three treatments; they were respectively 8.9 per cent., 3.0 per cent., and 8.6 per cent. higher than the observed ones. These percentages did not seem high enough to warrant the exclusion of these data from the regression.

It was also considered possible that, where asparagin was supplied to the plants directly, the relation between A_T and P would be distorted. Equation (4) was therefore fitted excluding the data for the asparagin treatments; the observed and calculated values can be compared in Table VIII. There is a tendency for the asparagin treatments to show negative differences, also shown in the comparison for the regression on the complete data (Fig. 5); furthermore, the inclusion of the asparagin treatment data slightly decreases the value of V . It does not seem justifiable at the present stage, however,

to conclude that the same relationship does not apply to these data as to those for other treatments.

Individual comparisons of observed and calculated values in certain cases differ by greater percentages of P than in Experiment I; the goodness of fit

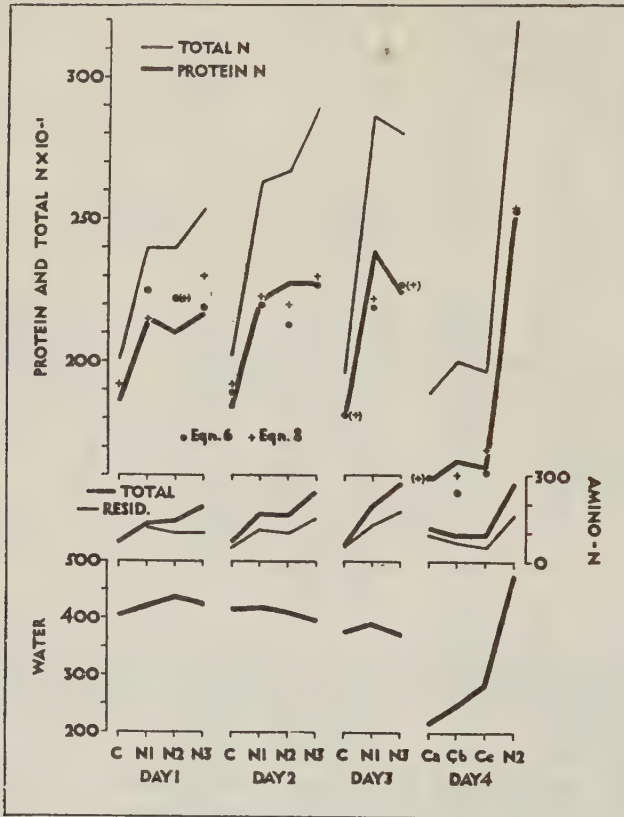


FIG. 4. Effect of ammonium sulphate treatments and varying water supply treatments on the contents of nitrogen compounds and water in the leaves, Experiment II. The nitrogen compound values are given in mg. per 100 gm. dry matter, and the water values in gm. per 100 gm. dry matter.

of the equations as measured by V , however, is greater: this is because the data extend over a greater range of values of P .

The observed and calculated values both exhibit the same general relationship to treatment, as shown in Figs. 4 and 5, even with such extremes of treatment as were given on day 4. The agreement is less good for the time drifts, but in this experiment these are of smaller magnitude than in the previous one, except in A_3 , and lie more within the limits of random variability of P .

Experiment III.

Time drifts. These can be considered only between days 1 and 2. The drift in U is small except for N_4 . For N_1 and N_2 , P follows the drift of A and the hydrolysis in N_4 may be associated with the fall in U . The drift of P in C and N_4 seems anomalous.

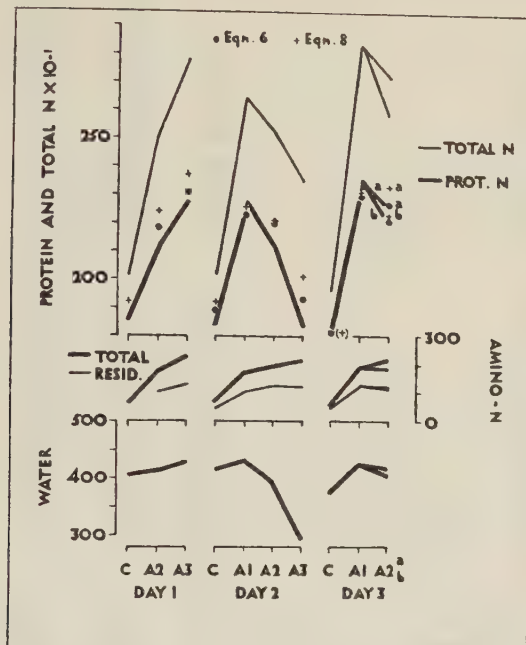


FIG. 5. Effect of asparagin treatments on the contents of nitrogen compounds and water in the leaves, Experiment II. The nitrogen compound values are given in mg. per 100 gm. dry matter, and the water values in gm. per 100 gm. dry matter.

Treatment effects. This experiment suffers again from the disadvantage of a marked negative correlation between U and A ; a greater range of A is, however, explored.

Regression functions. These are again given in Table VII and are based on the data for all three days. The values of V are lower than in the previous experiments. This may be partly due to the negative correlation between A and U , which tends to keep P constant, so that random variations in P constitute a comparatively large proportion of its total variance. It may also be partly due to the varying phosphorus supply. Figs. 6 and 7 show that the regression functions describe comparatively well the general treatment effects, but do not on the whole describe the drifts with time between days 1 and 2. Fig. 7 shows that $P_{calc.}$ is as a whole above $P_{obs.}$ on day 1 and below on day 3. Perhaps, then, in this experiment we are concerned with systems at different

TABLE VI
Results of Experiment III

	Treatment.				
	C.	N1.	N2.	N3.	N4.
DAY 1					
NH ₃ -N	10	19	157	710	926
2 × amide-N	139	245	502	598	886
Res. amino-N	108	190	324	452	433
Total amino-N	178	313	575	751	876
Residual N	83	23	17	85	210
Total sol. N	340	477	966	1675	2035
Protein N × 10 ⁻¹	209	220	221	205	213
Total N × 10 ⁻¹	243	268	318	373	417
Water	356	385	314	181	180
Red. sugars × 10 ⁻¹	311	417	478	695	772
Resp. rate.	3.5	4.0	3.7	2.7	2.9
DAY 2					
NH ₃ -N	10	17	188	670	801
2 × amide-N	127	412	629	750	1258
Res. amino-N	111	280	362	466	404
Total amino-N	175	486	677	841	1033
Residual N	67	15	110	196	233
Total sol. N	315	724	1069	1690	2230
Protein N × 10 ⁻¹	194	235	240	190	197
Total N × 10 ⁻¹	226	307	347	359	420
Water	365	368	280	172	135
Red. sugars × 10 ⁻¹	316	397	510	710	710
Resp. rate.	3.1	3.6	3.5	2.8	1.8
DAY 3					
NH ₃ -N	12	9	79	452	845
2 × amide-N	82	273	532	582	510
Res. amino-N	99	180	270	412	525
Total amino-N	140	317	536	703	780
Residual N	51	26	28	188	139
Total sol. N	244	488	853	1258	1741
Protein N × 10 ⁻¹	210	227	228	217	215
Total N × 10 ⁻¹	234	276	313	343	389
Water	373	354	314	192	164
Red. sugars × 10 ⁻¹	307	318	515	558	692
Resp. rate.	3.5	3.9	4.3	3.0	1.8

Nitrogen fractions and reducing sugars in mg. per 100 gm. dry weight.

Water in gm. per 100 gm. dry weight.

Respiration rate in mg. CO₂ per gm. per hour.

distances from a steady state, or else there are drifting factors that have not been taken into account.

General conclusions.

The picture suggested by the data is that the net rate of formation of protein from amino-acids decreases with reduction in the water content, or in other words, that the net rate of hydrolysis increases. From this it is probably safe to conclude that, at the steady state, the amount of protein

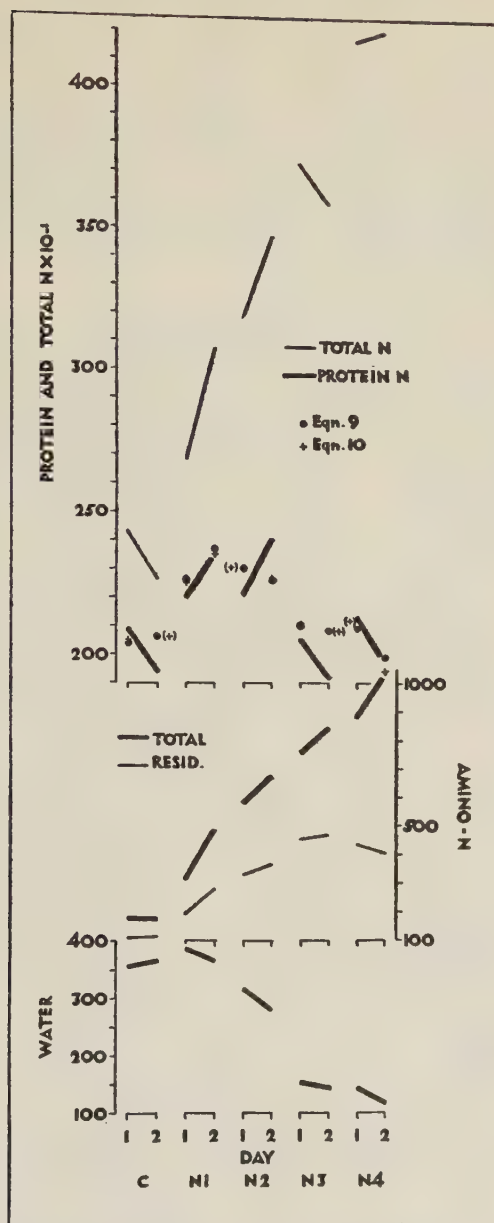


FIG. 6. Drifts with time in the contents of nitrogen compounds and water in the leaves, Experiment III. The nitrogen compound values are given in mg. per 100 gm. dry matter, and the water values in gm. per 100 gm. dry matter.

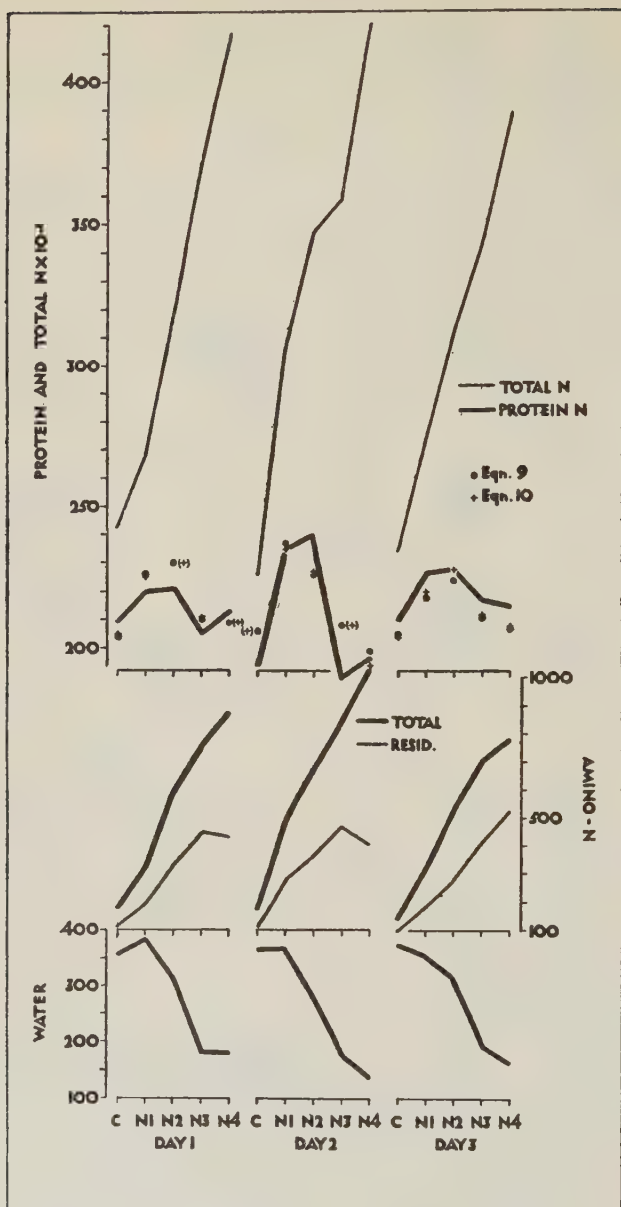


FIG. 7. Treatment effects on the contents of nitrogen compounds and water in the leaves, Experiment III. The nitrogen compound values are given in mg. per 100 gm. dry matter and the water values in gm. per 100 gm. dry matter.

TABLE VII

Regression Equations

All the regressions given are significant at or below the 5 per cent. point. Coefficients significant at or below the 5 per cent. point are given in bold type, those insignificant in italic type.

P = protein-nitrogen content, A_R = residual amino-nitrogen content, A_T = total amino-nitrogen content, U = water content, V = percentage of the variance of P ascribable to the average effect of the independent variables, D.F. = residual degrees of freedom, $\sqrt{\text{res. var.}}$ = square root of variance of P not accounted for by the regression.

	V	D.F.	$\sqrt{\text{res. var.}}$
EXPERIMENT I (omitting K_2SO_4 treatments).			
(1) $P = a + b_1 A_R + b_2 A_R^2 + b_3 U + b_4 pH$ $a = -284$ $b_1 = 6.85 \pm 1.707$ $b_2 = 0.0118 \pm 0.00392$ $b_3 = 1.79 \pm 0.282$ $b_4 = 190 \pm 66.3$	89.3	10	59.8
(2) $P = a + b_1 A_T + b_2 U + b_3 pH$ $a = -350$ $b_1 = 1.13 \pm 0.287$ $b_2 = 2.25 \pm 0.531$ $b_3 = 235 \pm 101.4$	74.5	11	92.6
EXPERIMENT II (omitting low water treatments).			
(3) $P = a + b_1 A_R + b_2 U$ $a = 918$ $b_1 = 3.50 \pm 0.630$ $b_2 = 2.09 \pm 0.682$	72.4	15*	183.9
EXPERIMENT II (omitting asparagin treatments).			
(4) $P = a + b_1 A_T + b_2 U$ $a = 833$ $b_1 = 2.55 \pm 0.361$ $b_2 = 2.20 \pm 0.356$	90.9	12	91.0
EXPERIMENT II (all treatments).			
(5) $P = a + b_1 A_R + b_2 U$ $a = 679$ $b_1 = 4.03 \pm 0.637$ $b_2 = 2.58 \pm 0.318$	89.3	18*	88.5
(6) $P = a + b_1 A_R + b_2 A_R^2 + b_3 U$ $a = 466$ $b_1 = 7.94 \pm 0.251$ $b_2 = 0.0186 \pm 0.0118$ $b_3 = 2.65 \pm 0.271$	92.4	17*	75.2
(7) $P = a + b_1 A_T + b_2 U$ $a = 796$ $b_1 = 2.29 \pm 0.293$ $b_2 = 2.46 \pm 0.284$	89.3	20	86.9
(8) $P = a + b_1 A_T + b_2 A_T^2 + b_3 U$ $a = 641$ $b_1 = 4.54 \pm 1.368$ $b_2 = -0.00699 \pm 0.00414$ $b_3 = 2.47 \pm 0.292$	90.2	19	83.2

* Treatment U of day 1 was omitted in calculating this regression.

TABLE VII (contd.)

EXPERIMENT III.	V	D.F.	$\sqrt{\text{res. var.}}$
(9) $P = a + b_1 A_R + b_2 A_R^2 + b_3 U$ $a = 1009$ $b_1 = 2.91$ $b_2 = 0.00296 \pm 0.00157$ $b_3 = 2.10 \pm 0.736$	48.4	11	100.3
(10) $P = a + b_1 A_T + b_2 A_T^2 + b_3 U$ $a = 1207$ $b_1 = 1.60 \pm 0.410$ $b_2 = 0.00107 \pm 0.000407$ $b_3 = 1.67 \pm 0.787$	56.6	11	95.1

TABLE VIII

P_{obs.} and P_{calc.} for Equation (4), Experiment II

Quantity ($\times 10^{-1}$)	Treatment.						
	C.	N1.	N2.	N3.	A2.	A3.	
DAY 1							
$P_{\text{obs.}}$	186	215	210	217	(212)	(228)	
$P_{\text{calc.}}$	190	209	215	225	(219)	(235)	
Diff.	-4	6	-5	-8	(-7)	(-7)	
DAY 2							
$P_{\text{obs.}}$	184	222	228	228	(228)	(211)	(184)
$P_{\text{calc.}}$	191	218	214	232	(220)	(217)	(201)
Diff.	-7	4	14	-4	(8)	(-6)	(-17)
DAY 3							
$P_{\text{obs.}}$	179	239	224	(236)	(225)	(219)	
$P_{\text{calc.}}$	181	219	234	(226)	(231)	(219)	
Diff.	-2	20	-10	(10)	(-6)	(0)	
DAY 4							
$P_{\text{obs.}}$	158	165	163	256			
$P_{\text{calc.}}$	159	160	169	258			
Diff.	-1	5	-6	-2			

present increases both with the amino-acid content and with water content. The goodness of fit of the regression functions would be accounted for if the system was near a drifting steady state during the course of the experiments. It is not, however, safe to conclude that the precise form of the regression is of significance; thus the significant positive coefficient of pH and negative of A^2 may be fortuitous, and the result of fitting the data to arbitrary functions of the other variables.

It is noteworthy that significant partial regression coefficients for respiration rate could not be obtained. Smirnow (1925) has concluded that there is a relation between protein content and respiration rate, but he did not determine partial relationships.

Concentration Relationships between Proteins and Amino-acids

In suggesting an interpretation for the effect of water content on the steady-state relation between the contents of proteins and amino-acids, there are two

possibilities: firstly, the change in water content may produce its effect merely by changing the concentrations of the proteins and amino-acids; secondly, the change in water content may specifically affect the rate of some reaction or reactions in the system, or else may be a measure of some other factor that does so.

Let us consider the first possibility. Wasteneys and Borsook (1930) have suggested that in the living cell a true equilibrium between proteins and amino-acids is probably closely approached, the equilibrium being characterized by the equation

$$[P] = k[A]^n,$$

where $[P]$ and $[A]$ are the concentrations of proteins and amino-acids, and n is the number of amino-acids in one molecule of protein. It would follow from this equation that the curve relating $[P]$ to $[A]$ is convex to the $[A]$ axis.

It seems, however, unlikely that such an equilibrium occurs in living cells; it is more probable that there is a mechanism, involving expenditure of energy, that causes the concentration of proteins at a steady state to be greater than that of equilibrium. Also a portion of the protein¹ may be so changed as to have no direct relationship to the concentration of amino-acids present; and the relation between the two protein fractions may be dependent on metabolic activity.

With such a system we cannot forecast the form of the steady state relation between the concentrations of total proteins and amino-acids; we can, however, consider the consequence of the various forms the relation might possess. If

$$[P] = k[A],$$

clearly a change in water content, provided such change has no effect on the proteins and amino-acids other than changing their concentrations, will have no effect on the amounts of these substances in the leaves. Suppose, however, the relation is such that, with increase of concentrations of these substances from one pair of steady state values of $[P]$ and $[A]$ to another,

$$\frac{\Delta[P]}{\Delta[A]} > \frac{[P]}{[A]},$$

in this case a reduction in water content will result in a quantity of protein being synthesized before the new steady state is attained. If, on the contrary,

$$\frac{\Delta[P]}{\Delta[A]} < \frac{[P]}{[A]},$$

hydrolysis of protein will occur. The latter inequality could exist if the $[P]$ - $[A]$ curve is concave to the $[A]$ axis, or if it is convex over a limited range of values of the variables; it could also exist if the curve were always convex, provided that $[P]$ has a positive value when $[A]$ is zero, and that the amount

¹ Smirnow and Iszwochikow (1930) have produced evidence that there is a stable protein fraction that does not enter into the metabolic flux.

of protein represented by this value is capable of entering into the protein amino-acid flux.

The data before us are insufficient to enable us to decide which of these forms characterizes the relationship between protein and amino-acid concentrations; we have no measure of $[P]$ and $[A]$, especially as U is at best only an anamorphic measure of the amount of water in the phases in which proteins and amino-acids occur. The fact that the coefficient of the A^2 term in the regression equations is negative suggests that the $[P]$ - $[A]$ curve may be concave to the $[A]$ axis; but in view of the complexity of the variations in the system no great significance can be placed upon the precise form of these equations.

Clearly, however, the system may be such that the amount of protein can increase with increase in amounts of both amino-acids and water, the water effect being purely the result of a certain form of the relation between the steady state concentrations of protein and amino-acids; the protein content would thus be determined by water content only in so far as the latter determines the concentrations of amino-acid.

The second possibility is that the change in water content *per se* affects the velocity constants of protein synthesis and hydrolysis. Possibly reduction in water content may decrease the rate of synthesis. In each experiment, in the treatment where no nitrogen has been added, hydrolysis takes place, in Experiments I and II to an extent not to be expected from the regressions; this may be a normal ontogenetic drift. When extra substrate is added, synthesis occurs at a rate dependent on the water content, so that at very low water contents it is outweighed by the normal process of hydrolysis.

This second possibility implies that there is not a single $[P]$ - $[A]$ relation, but that this relation is dependent on the water content.

The Work of Mothes

Prior to the carrying out of the present investigation it had been shown by Mothes (1931) that reduction of the water content of leaves was accompanied by protein hydrolysis. But it is not clear from his experiments whether this effect is reversible, or whether there is a steady-state relationship between the amounts of proteins, amino-acids, and water. Only one set of results (p. 708) is quoted in which reversibility is suggested, but the data are insufficient to show that fluctuations in water content are accompanied by corresponding fluctuations in protein content; the changes produced in mature and old leaves were irreversible. In the present work there is definite evidence that the effect is a reversible one (cf. Fig. 1).

Furthermore, except in one experiment, Mothes did not determine the amount of amino-acids, and in this experiment the effect of water content is superimposed on a starvation effect. Mothes was therefore unjustified in assuming from his results that the effect was necessarily on the protein amino-acid relation: it could also have been due to an effect on the relation between

the amounts of amino-acids and of their precursors. The present investigation excludes the latter effect as the major factor.

In a further work Mothes (1933) showed that, when protein hydrolysates were injected into leaves in the dark, net protein synthesis occurred in pure oxygen, no appreciable change occurred at 20 per cent. oxygen concentration of the atmosphere, and net hydrolysis occurred at lower concentrations. In narcosis experiments he claims to have shown that high partial pressures of oxygen inhibit protein hydrolysis whereas oxygen deficiency inhibits synthesis. Mothes concludes that in wilting leaves it is the closure of the stomata and consequent oxygen deficit in the leaves that causes protein hydrolysis. When wilted leaves were cut to enable oxygen to penetrate, proteolysis was inhibited. He refers to the evidence that proteolytic enzymes are activated by reduced sulphhydryl compounds and suggests that the production of these in the leaves in oxygen deficiency leads to the protein hydrolysis.

Mothes' work has been criticized by Paech (1934, 1935), who found that, in the absence of oxygen, hydrolysis usually does not begin until there are definite signs of injury to the tissue. It occurs prior to this only if injury is very late in occurring, in which case carbohydrate exhaustion is the cause. Injury and hydrolysis are delayed if the tissue is rich in carbohydrates. Paech points out also that both Mothes' low oxygen plants and his narcotized plants were injured.

Alternative Hypothesis of the Stages in Protein Synthesis

Our considerations so far have been based on the assumption that the proteins are synthesized solely from, and hydrolysed back to, amino-acids. There is, however, the alternative hypothesis that proteins are derived from some other compounds, and that the amino-acids arise only by protein hydrolysis. It is obvious that the data could be fitted by regressions of the type

$$A = f(P) - \phi(U)$$

which would conform to the conception of reduction in water content causing increased hydrolysis or decreased synthesis of proteins. But we cannot investigate satisfactorily the factors that might determine the amounts of protein present since we have no measure of the supposed antecedent carbon compounds; there is, however, a positive correlation between ammonia-nitrogen and amino-acid contents, so that P could probably be fitted to regressions on ammonia-nitrogen and U .

Change in Water Content Associated with Treatment

It is apparent that, while in all experiments high supplies of ammonium sulphate are associated with low water contents of the leaves, such association is much less evident for similar supplies of potassium sulphate. The lowered water content is therefore probably not the immediate result of the difference

in osmotic pressure between the external solution and the sap of the root-cells, but is more probably a specific effect of ammonia or the NH_4 -ion.¹ Mevius (1928) suggests that in high concentrations of ammonium salts the rapid intake of ammonium radicle causes a toxic alkalinity in the sap of the roots; this might reduce the rate at which water could be supplied to the leaves and so lead to reduction of their water content. High concentrations of ammonia nitrogen in the leaf in the present experiments have produced no appreciable increase in the pH of the expressed leaf sap, which suggests that the buffering capacity of the leaf sap must be very high.²

From its position in the Hofmeister series it might be expected that the NH_4 -ion might decrease the swelling capacity of cytoplasmic colloids, but it is unlikely that this would have much effect on the total water content of the tissues.

The results of Experiment II show that the relation between protein and water content is similar whether the low water content values are produced by high nitrogen supply or by deliberately restricted water supply. While, however, we have so far assumed that the water content is a determinant of protein content, it is possible that the protein content is also a factor determining the water content to some extent, since with the first increases in nitrogen supply there is frequently an increase in both protein nitrogen and water content.

SUMMARY

This paper is an account of an investigation of the relation between the amount of protein in the leaves of plants and certain other quantities which varied as the result of experimental treatment of the plants. The results suggest that, under environmental conditions constant in certain respects, the protein content of the leaves increases with both amino-acid content and water content, and in one experiment there was evidence that it increased with the pH of the expressed sap also. The picture suggested is that the net rate of formation of protein from amino-acids decreases with reduction in water content, although we cannot tell whether synthesis is decreased or hydrolysis is increased, or whether both are affected. It seems probable that, at the steady state, the amount of protein present increases both with amino-acid content and with water content. The effect of water content on the protein amino-acid relation may be merely the result of change in the concentrations of these two components of the system; change in water content may, on the contrary, affect the rate of some reaction or reactions in the sequence, or else it may be a measure of some other factor that does so.

It is shown incidentally that increasing the supply of NH_4 -ions to the roots

¹ A similar difference is exhibited in the effects of nitrogen and potassium on growth (Rippel and Meyer, 1933; Rippel, Behr, and Meyer, 1933).

² Theron (1924) and Keyssner (1931) showed that the pH of the external medium affected that of the root sap, but had little effect on that of the shoot sap.

of a plant causes, over a certain range, marked reductions in the water content of the leaves.

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Excretion of Nitrogenous Substances from Leguminous Root Nodules: Observations on Soya Bean

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I. INTRODUCTION¹

IN a previous investigation (Bond, 1936) certain observations were made on the rate of transfer of fixed nitrogen from the nodule-bacteria of soya bean to the host plant over successive periods in the life-cycle of the latter, and on the relation of this rate of transfer to the rate of fixation of nitrogen. It was pointed out how some uncertainty existed as to the completeness of the figures presented, owing to the demonstration by Virtanen and his collaborators (see Virtanen, 1935, and Virtanen, von Hausen, and Laine, 1937) that in some legumes, notably the pea (*Pisum sativum* L.), there may be a very considerable loss of fixed nitrogen through the occurrence of leakage or excretion from the nodules into the rooting medium. In showing this Virtanen has confirmed a belief held by some earlier investigators, especially Lipman (1912), although the matter had been largely lost sight of until the appearance of Virtanen's work. The experiments to be reported in the present paper were undertaken with the object of detecting and measuring any such excretion from nodules of soya bean under cultural conditions similar to those obtaining in the author's previous experiments. In addition it is a matter of general botanical interest to obtain as much information as possible about this excretion from leguminous nodules, in view of the importance which the phenomenon may assume in mixed plant populations, wild or cultivated, in which legumes are included.

Lipman (loc. cit.), who appears to have performed the first experiments on the associated growth of legumes with non-legumes, included some observations on soya beans and recorded that while with some legumes the non-legume benefited from the association, in the case of soya bean (and cowpea)

¹ Preliminary statements regarding the present work have already appeared (Bond, 1937 *a, b*).
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the benefit to the non-legume was much less marked. A different conclusion was reached by Stallings (1926), who deduced from his experiments with pot cultures that wheat may obtain considerable amounts of nitrogen from noduleated soya beans. His work is considered in detail in the third section of the present paper. In a preliminary note Wilson (1937) reports his inability to detect excretion in pot cultures of soya bean. There is also some literature on the growth of soya bean and cereal mixtures under field conditions, references being given in a review by Nicol (1936). It suffices to say here that such field experiments have given no clear evidence for or against uptake of nitrogen by the cereal associate from the legume. The position with regard to excretion from soya bean nodules is thus one of considerable obscurity.

In the present work, evidence of excretion has been sought along two lines, namely, (a) by determination of the nitrogen content of sand in which noduleated soya beans have been grown, and (b) by examination (for nitrogen uptake) of non-leguminous test plants grown in the same pots as soya beans, barley being used for this purpose. Virtanen (1935) has shown that barley plants are capable of direct utilization of a substantial part of the nitrogenous excretion from pea nodules.

II. METHODS

Cultural arrangements.

Arrangements in general were identical with those of the previous investigation (Bond, 1936), except for a few modifications which seemed likely to benefit plant development.

The plants were grown in the glass-houses in sand culture, glazed earthenware jars without drainage-holes being used, of capacity varying from 2.3 to 3.6 kg. of sand in different experiments. A quartz sand of medium fineness was employed and was washed in a current of tap-water for twenty-four hours before use. In some experiments the sand was ignited after washing, in order to purify it further. As the sand was weighed out into the pots, calcium carbonate was mixed in at the rate of 2 gm. per kg. of sand, pots and sand being subsequently autoclaved at 15 lb. pressure for three hours. Sterile culture solution was then added to each pot until the weight of the latter indicated that 12 per cent. of moisture was present, reckoned on the weight of dry sand. The culture solution, based on that of Hiltner with nitrate omitted, had the following composition: $\text{Ca}_3(\text{PO}_4)_2$, $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, and KCl , 0.25 gm. each; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.39 gm.; FeCl_3 (5 per cent. solution), 3 drops; water, 1 litre. This solution had an initial pH of 6.5, but no pH measurements were made on the sand in which the plants were growing.

The 'Manchu' variety of soya bean was again used, the seeds being kindly supplied by Dr. W. J. Morse of the Bureau of Plant Industry, Washington. Sound seeds of definite weights were selected, and were initially surface-sterilized by being immersed in absolute alcohol for ten seconds and then passed through a flame. After being imbibed, the seeds (except in the control

pots) were inoculated with a particular strain of *Bacillus radicola*¹ and sown. Every precaution was taken to prevent contamination of the plant cultures by foreign organisms during the experiments. Every second or third day the moisture content of the sand was restored to 12 per cent. by the addition of sterile water or occasionally of further sterile culture solution.

In the case of the mixed culture experiments the procedure, apart from the introduction of the barley, was as above. The barley grains (variety Spratt Archer, of selected weight 0.055 to 0.06 gm.) were initially sterilized by successive immersion in absolute alcohol and 0.2 per cent. mercuric chloride for two minutes in each, followed by several rinses in sterile water, a method similar to that of von Hausen (1936).

Sampling and nitrogen estimations.

Sample pots of inoculated and control plants were taken from time to time. In the case of the experiments involving analysis of the rooting medium the procedure with each pot was as follows. The contents of the pot were tipped out and the plant root systems rinsed in 200 c.c. water in order to wash off any excreted substances that might be present on the surface of the nodules (in the case of an inoculated pot) or in the sand still adhering to the roots. This water was combined with an additional portion used to rinse out the pot, and after the addition of further washings (see below) the whole was acidified with sulphuric acid, evaporated down almost to dryness under reduced pressure, and the residue was submitted to Kjeldahl analysis. The plants were dried and total nitrogen contents determined by the salicylic acid-Kjeldahl process as modified by Ranker (1925, 1926).

In experiments I and II the sand from the culture pot was spread out on an enamelled tray and dried at a temperature of 30° to 35° C., root fragments being then picked out and the sand thoroughly mixed by hand. Four 200-gm. portions of the sand were then weighed out for analysis and transferred to 800 c.c. Kjeldahl flasks, the residual sand being removed and the tray rinsed with water. This water was combined with the previous wash-water.

In experiments III and IV the preliminary drying of the sand was omitted in view of the possibility that in the case of the soya bean the excreted substances, if any, might be volatile. Immediately after tipping out, the sand was thoroughly mixed and its total weight ascertained. Four 200-gm. portions were then taken and transferred to Kjeldahl flasks, after removal of root fragments.

The nitrogen content of the sand portions was determined by a method which was essentially the salicylic acid-Kjeldahl process referred to above, with certain modifications resembling in some respects those adopted by Virtanen and von Hausen (1935). Eighty c.c. of the acid mixture was added to each 200-gm. portion of sand, and after combustion and subsequent

¹ The writer is greatly indebted to Dr. I. L. Baldwin, University of Wisconsin, for supplying bacillus cultures.

dilution with 200 c.c. water the liquid contents of the flask were filtered off from the sand, using a Buchner funnel fitted with acid-resisting paper. The sand on the filter was washed with a further 500 c.c. water. Halves of the four filtrates thus obtained in connexion with a pot of sand were measured out and combined in pairs, the two resultant mixtures, each corresponding to a composite 200-gm. portion of sand, being evaporated down to a volume of 350 c.c. Each was then made alkaline and distilled over in the usual way into N/50 HCl. The figures obtained on subsequent titration of the contents of the two receiving flasks with N/50 NaOH always agreed closely, and from the average of the two titrations, after suitable allowance for blank estimations (reagents alone), the nitrogen content of the whole of the sand from the culture pot under examination was calculated. The figure so obtained, to which any nitrogen found in the combined washings was added, was thus based on the examination of 800 gm. of the total sand in a pot, which varied from 2.3 to 3.6 kg. in different experiments.

This procedure was thoroughly tested in preliminary estimations on sand to which small quantities of nitrogen had been added as ammonium chloride or asparagine. Weighed amounts of these substances in a finely powdered form, representing 30 mg. to 100 mg. nitrogen in different trials, were added to 3 kg. lots of sand of known nitrogen content and thoroughly stirred in by hand. In each trial four 200-gm. samples of the sand were taken for analysis. Recoveries of 90 to 93 per cent. of the added nitrogen were repeatedly obtained and were considered satisfactory under the rather difficult circumstances. These trials showed not only that the modified Kjeldahl process was satisfactory but also that the examination of 800 gm. of sand from a total of 3 kg. (which approximates to the content of a pot in the actual experiments) could be relied on to give an accurate estimate of the nitrogen content of the whole of the sand, into which the added nitrogenous substances had been stirred by the same procedure as was used for mixing the sand in the experiments proper.

It was recognized at the time that the use of moist sand in the nitrogen estimations of experiments III and IV was open to the objection that any nitrate-nitrogen present might be at least partly lost (see Ranker, *loc. cit.*). From the negative results of experiments I and II (see later) it was concluded that the chance of nitrate-nitrogen being present could be dismissed. The use of the salicylic acid variation of the normal Kjeldahl process was continued since this method had been employed in all preliminary tests.

III. DATA

(a) *Sand analysis experiments.*

The figures for the first two experiments, which were conducted in 1936, are presented in Tables I and II.

As indicated in the tables, a certain amount of combined nitrogen was initially present in the sand as impurity, consisting almost certainly of peaty

material retained from the large volume of tap-water used in washing the sand. Experience has proved that this nitrogen is unavailable to plants and remains in the sand unabsorbed throughout the experiment, its presence being thus no serious detriment.

TABLE I

Experiment I. Bacillus Strain Wisconsin No. 9. Four Soya Bean Plants per Pot containing 3.6 kg. Sand. Figures for Inoculated Pots are Averages of Two Pots, those for Controls being based on Single Pots. Original N Content of Sand = 20 mg. per Pot. N Content of Four Seeds = 38 mg.

Date of sample.	Age in days.	N content of four plants (mg.).	N content of sand (mg. per pot).
Aug. 5	41	60	21.5
Aug. 20	56	—	21.5
Sept. 2	69 ¹	—	23.5
Sept. 16	83 ¹	—	25.0
Oct. 1	98	—	26.0
Oct. 20	117 ²	211	24.5
<i>Control pots (plants non-nodulated).</i>			
Sept. 16	83 ¹	44	25.0
Oct. 20	117	44	25.0

¹ Flowering.

² Pods full.

TABLE II

Experiment II. Bacillus Strain Wisconsin No. 9. Four Soya Bean Plants per Pot containing 2.5 kg. Sand. Figures for Inoculated Pots are Averages of Two Pots. Original N Content of Sand = 17.5 mg. per Pot. N Content of Four Seeds = 47 mg.

Date of sample.	Age in days.	N content of four plants (mg.).	N content of sand (mg. per pot).
Aug. 21	41	59	19.0
Sept. 4	55	90	19.5
Sept. 21	72 ¹	130	20.0
Oct. 9	90	153	21.0
Oct. 26	107 ²	174	24.5

Control pots (non-nodulated).

Oct. 9	90	{ pot A	54	20.5
		{ pot B	53	

¹ Flowering.

² Pods ripening.

It will be seen that during the course of the experiments the nitrogen content of the sand increased slightly, both in the inoculated pots and in the controls. In the first experiment the final value of the increase with the inoculated pots was the same as that in the control pots. In the second experiment the final inoculated pot showed a slightly greater increase over the original nitrogen content of the sand than in the previous experiment, though the absence

of a control pot for this final date makes it impossible to say how far this late rise would have proved peculiar to inoculated pots. These two experiments furnish no evidence for anything more than negligible excretion from the nodules. It should be stated that the figures for the two pots of inoculated plants taken on each sampling date were always practically identical.

Fixation of nitrogen, determined by subtracting seed nitrogen from plant nitrogen, finally amounted in the first experiment to 173 mg. and in the second to 127 mg., in both cases for four plants. As regards nodulation, forty nodules per pot of four plants were present at the first sampling in experiment I, while by September 16 the number had increased to 100, with a fresh weight of 1 gm. Nodulation in the second experiment was very similar.

One feature calling for explanation is that the plant nitrogen in all control pots exceeded that of the original seeds. In these two experiments the plants were supplied with sterile tap-water, since the distilled water available had proved slightly unsuitable for soya beans. It is evident that the water contained traces of available nitrogen, and hence the slight gain in nitrogen content shown by the control plants. This gain was never detected when distilled water was used (see experiments below).

Table III gives the results of a third experiment in which the nitrogen estimations were conducted on the moist sand without previous drying (see Methods). *Bacillus* strain No. 9 was again used. This experiment was commenced in the early spring of 1937, and for the first three months natural light was supplemented by the illumination from a 1000-watt lamp suspended 2 to 3 feet above the plants, which were given a fourteen-hour 'day'. Growth was superior to that previously obtained in summer months with natural light alone. Fixation was also greater, and amounted to some 350 mg. for five plants, this being probably due in part to the fact that for several weeks all flower-buds were removed from the plants.¹ At the final sampling date, 160 nodules, with a fresh weight of 2.5 gm. were present on the five plants of the pot.

As in experiments I and II a slight increase occurred in the nitrogen content of the sand in both inoculated and control pots during the course of the experiment. The increase in the case of the inoculated pots finally exceeded that in the controls by 2.5 mg. only. In this and the subsequent experiments a supply of glass-distilled water was used for the cultures, and it will be observed that the nitrogen content of the control plants never exceeds that of the original seeds by a significant amount.

A fourth experiment, in which ignited sand practically free of nitrogen was used, was also carried out, employing two other strains of soya bean bacillus from the Wisconsin collection, Nos. 505 and 17. The results are presented in Table IV. These plants also received artificial illumination, but growth and fixation were inferior to those in the previous experiment, partly owing to the

¹ This point is significant in view of the suggestion of Wilson (1937) that in peas excretion is favoured by a delayed reproductive phase.

use of smaller pots, and also, in the case of the plants infected with strain No. 17, to the known low efficiency in fixation of this strain. Strain No. 505 is one of the most efficient strains in the Wisconsin collection, as is also No. 9,

TABLE III

Experiment III. Bacillus Strain Wisconsin No. 9. Five Soya Bean Plants per Pot containing 3.6 kg. of Sand. Single-pot Samples. N originally present in Sand = 17 mg. per Pot. N Content of Five Seeds = 77 mg.

Date of sample.	Age in days.	N content of five plants (mg.).	N content of sand (mg. per pot).
March 25	53	120	17.0
April 7	66	140	17.0
April 22	81	170	17.0
May 8	97 ¹	230	20.0
May 24	113 ¹	310	20.5
June 26	146 ²	430	24.0
<i>Control pots (non-nodulated).</i>			
April 7	66	78	16.0
May 24	113 ¹	79	16.5
June 26	146	79	21.5

¹ Flowering.

² Pods full.

TABLE IV

Experiment IV. Bacillus Strains Wisconsin Nos. 505 and 17. Three Soya Bean Plants per Pot containing 2.3 kg. Sand. Single-pot Samples. Original N Content of Sand = 3 mg. per Pot. N Content of Three Seeds = 46 mg.

	Date of sample.	Age in days.	N content of three plants (mg.).	N content of sand (mg. per pot).
Inoc. Strain 505.	June 2	80 ¹	106	8.0
	July 7	124 ²	190	8.0
Inoc. Strain 17.	June 2	80 ¹	61	8.0
	July 7	124 ²	89	16.0
	July 17	134 ²	126	11.0
Controls	July 17	134	39	13.0
	July 17	134	—	12.0

¹ Flowering.

² Pods ripening.

used in the previous experiments. The nitrogen contents of the sand from inoculated and control pots increased to about the same extent in each, the increases tending to be greater than in the previous experiments.

(b) *Soya bean with barley cultures.*

Table V gives results for an experiment in which beans and barley were sown at the same time. The barley soon developed features of nitrogen starvation and growth was arrested at the point of ear-emergence. No difference in appearance was detectable between those barley plants associated with nodulated beans and those with the control, non-nodulated beans.

Analysis confirmed that the barley plants had absorbed no nitrogen during their growth, the nitrogen contents showing no appreciable difference from that of the original grains. Fixation in the nodulated soya beans amounted finally to 247 mg. for three plants (pot 3).

TABLE V

Soya Bean and Barley Cultures, set up Feb. 20, 1937, Three Soya Beans and Two Barley Plants per Pot, containing 3.6 kg. Sand. Bacillus Strain Wisconsin No. 9. N Content of Three Soya Bean Seeds = 46 mg., and of Two Barley Grains = 1.8 mg.

	Pot no.	N content of the three soya bean plants (mg.).	N content of the two barley plants (mg.).
Soya beans nodu- lated	1 ¹	154	2.3
	2 ²	254	1.6
	3 ²	293	1.8
Soya beans non- nodulated	1 ¹	48	2.3
	2 ²	48	1.4

¹ Plants taken out for analysis June 1.
² „ „ „ „ July 1.

In a further experiment the soya beans were sown on February 4, but the barley was not sown until March 17, by which time the beans had made considerable growth and fixation had commenced. After a further period of three months the nitrogen contents of barley plants from two pots containing nodulated beans were 1.6 mg. and 1.7 mg. respectively, for two plants in each case, while in the control pot the corresponding figure was 1.9 mg. Thus, again, there was absolutely no trace of nitrogen uptake by the cereal. Fixation by the soya beans here was 320 mg. for five plants.

While in the preceding mixed cultures bacillus strain No. 9 was used, precisely similar results were obtained in an experiment in which strain No. 505 was employed. The fixation by the soya beans was 175 mg. for three plants.

IV. DISCUSSION

The tables in the preceding section show that while, in the experiments involving analysis of the sand in which nodulated soya beans had been grown, an increase of a few milligrammes (itself quite negligible compared with Virtanen's figures) was regularly obtained in the nitrogen content of the sand, a similar or slightly smaller increase was also detected in the sand from the control pots, with non-nodulated plants. Factors productive of an increase in sand nitrogen in the control pots were presumably operative also in the inoculated pots, and it is, of course, only when the increase in the latter pots exceeds that in the control pots that evidence for excretion is forthcoming. On the basis of these results there is thus no evidence for anything more than negligible excretion from the nodules of the inoculated plants. The origin of

the small increase in the sand nitrogen of the control pots is considered later in this section.

It is recognized that analysis of the rooting medium may give a misleading impression of the extent of any excretion that may be in progress, since some reabsorption of the excreted substances by the legume roots may occur, reducing the extent to which the substances *accumulate* in the sand. Virtanen, von Hausen, and Laine (1937) have described a preliminary experiment suggesting that frequent watering, necessary in open cultures, may assist such a process of reabsorption, although in that experiment accumulation was still considerable. It can hardly be imagined, in the event of appreciable excretion occurring, that reabsorption could be so complete and instantaneous that no trace of the excreted substances would be found in the sand at any stage in the experiments, which was practically the result obtained by the present writer. It is especially significant that the nitrogen content of the combined wash-water (see Methods), which included the water in which the roots and nodules were rinsed, never amounted to 1 mg. If excretion were proceeding, even though accompanied by reabsorption, one would expect to find definite evidence of it in the root-washings.

Excretion, even though accompanied by reabsorption, should also be detected by the second type of experiment, involving examination (for nitrogen uptake) of non-leguminous test plants grown in the same pots as the legumes, since the roots of the test plant, closely intermingled with those of the legume, should themselves absorb a proportion of the excretion. Any factor, such as watering, which assists legume reabsorption should also assist absorption by the test plant. As reference to the results will show, there was no sign of any uptake of nitrogen by the barley plants of the present experiments. So far as the evidence of these mixed cultures goes, it might be suggested that excretion was proceeding in these experiments and that the excreted substances, unlike those from pea nodules, were unsuited for absorption by barley. In view of the negative results from the sand analysis experiments this possibility appears to be very remote.

It is, then, very improbable that any appreciable excretion took place in the experiments here described. There are two possible reasons for this negative result: (a) that some at present unidentified condition, necessary if excretion is to proceed, was not provided in these experiments, or (b) that excretion never occurs in soya bean, at least, not in the 'Manchu' variety when infected with any of the three bacterial strains employed here.

Further work is required before the matter can be taken further. Our ignorance of the factors affecting excretion is illustrated by the experiences of Wilson (1937), who points out that in the pea excretion may be obtained under one set of conditions, while under other not obviously very dissimilar conditions excretion may be absent. The circumstance that the plant cultures of the present author's experiments were open to the atmosphere and, therefore, are not to be described as sterile (though all materials were initially sterilized),

is not in itself likely to be productive of a negative result. Virtanen (1936) has obtained excretion with the pea in both closed, sterile cultures and in open cultures, so that clearly complete sterility is not essential to the detection of excretion phenomena, and, indeed, there is no obvious reason why it should be. A possible suggestion is that excretion may only occur when fixation is so rapid that an excess of fixation products accumulates in the nodule tissues, and the required rate may not have been attained in the experiments considered here. Actually it appears that the rate of fixation is not a deciding factor, for Virtanen, von Hausen, and Laine (1937) report excretion from peas with very poor fixation, while Wilson (1937) also finds that the occurrence of excretion in peas is not dependent on the amount of fixation. It may, however, be noted that comparison with figures given by other investigators for soya bean (see Wright (1925), Eaton (1931)) suggests that the fixation obtained in the author's work, especially in experiment III and in some of the mixed cultures (Table V), was good for pot cultures, though naturally much inferior to results in the field. Thus Wright (*loc. cit.*) obtained a fixation of 330 mg. per plant of 'Manchu' soya bean under field conditions, four times greater than the maximum fixation in the present work.

It is necessary to consider here the work of Stallings (1926), whose experiments in pot culture were concluded to show that wheat plants may obtain considerable amounts of nitrogen, possibly in the form of ammonia, from nodulated soya bean plants when the two are grown in association. This conclusion was based chiefly on a consideration of the relative ammonia contents of wheat plants grown alone, and of others grown with soya beans. Consideration of absolute nitrogen content of the wheat plants would be a more satisfactory procedure in testing for nitrogen uptake, but the matter is complicated in Stallings' experiments by the initial presence in the soil used of appreciable amounts of available nitrogen. The following absolute figures have been calculated from the dry matter and percentage nitrogen data furnished by Stallings, all figures being milligrammes per plant and applying to the second harvests:

	N content of wheat grown alone.	N content of wheat grown with soya beans.
Series I (fertile soil)	88	59
„ II (poor soil)	22	34
„ III (poor soil sterilized) . .	42	34

In one experiment only was the nitrogen content of wheat grown with soya beans higher than that of wheat grown alone, while in the other two cases it was actually lower. It is not, however, certain that the wheat plants in the two sets of pots (wheat alone, wheat plus soya beans) had equal opportunities of absorbing the nitrogen initially in the soil, so that very much stress cannot be placed on these figures, and the experiments are inconclusive.

In the same paper Stallings reports analyses of sand in which nodulated

soya beans had been grown. The final nitrogen content of the sand is given in Table 25 as 0.015 per cent., which is high under the circumstances, but since it is not clear whether this figure applies to the whole of the sand in the pot or only to that near the roots, the absolute nitrogen content of the pot of sand cannot be calculated, and in the absence of figures for the control pots no comparison with the latter pots is possible. Amino-acids were absent from the sand, but traces of ammonia and nitrates were detected, recalculation indicating that less than 0.5 mg. of the former substance was present per pot. These experiments cannot be said to prove that leakage of nitrogen occurred from the nodules.

The position is, then, that so far there is no clear evidence of excretion from nodules of soya bean, though it remains to be seen how far subsequent work changes this position. There is no immediately obvious reason why the soya bean should differ from other legumes in the matter of excretion, though the structure of the peripheral tissues of leguminous nodules may prove to be a determining factor in excretion. In soya-bean nodules a practically complete layer of fibrous cells is present from an early stage, enclosing the bacterial tissue. These cells, however, are not suberized and do not appear likely to prevent leakage. Suberization of more peripheral cells does occur and may preclude leakage from older nodules, but the position in young nodules has not yet been studied.

Since the experiments described in this paper were carried out with a similar procedure and under comparable conditions to those of the previous investigation (Bond, 1936), the same variety of soya bean and identical bacillus strains being used,¹ it is concluded that in the earlier experiments also there was no excretion. The measurements of fixation and transfer of nitrogen made there thus appear to be complete in themselves and to require no amendment on account of excretion, the differentiation between 'apparent' and 'real' fixation (p. 561) being unnecessary. The conclusions drawn from the earlier investigation do not require any reconsideration, since, as was explained at the time, they were not materially affected by the question of whether or not excretion had occurred. It may be mentioned that the necessary analyses were made in connexion with experiment II, of the present series of experiments, to permit of the calculation of fixation and transfer of nitrogen as in the previous work, the figures being given in Table VI. The figures are very similar to those reported in the previous paper. In the final period transfer exceeded fixation by a small amount, but it is possible that this tendency, also detected in the earlier experiments is significant, though not then thought to be so.

It remains to consider the origin of the small increase in sand nitrogen observed in all control pots of experiments involving sand analysis. The increase amounted to 10 mg. in the case of one pot in experiment IV, but in

¹ In the previous work referred to, Wisconsin bacillus strain No. 505 was used in the 1932 experiment, No. 17 in 1934, and No. 9 in 1935.

the other experiments it did not exceed 5 mg. As previously remarked, one assumes that whatever the factors productive of this increase, they were also operating to the same extent in the corresponding inoculated pots. Trial experiments showed that an *apparent* increase in sand nitrogen up to 2 mg. per pot arises through unavoidable inclusion in the analysed sand of small

TABLE VI

Fixation and Transfer of Nitrogen during Successive Periods in Experiment II. Figures based on Examination of Four Pots on each Date, each Pot containing Four Plants

Period (days from sowing).	N fixed by bacteria of 4 plants (mg.).	N transferred into 4 plants from their nodules (mg.).	Ratio of N transferred to N fixed, as % of N fixed.
41-55	31	26	84
55-72	41	38	93
72-90	25	22	88
90-107	12	15	124

root fragments, broken off during sampling. The residual (actual) increase may be due to (a) sloughing-off of peripheral root-tissues, and/or (b) contamination of the cultures with asymbiotic nitrogen-fixing bacteria. The former process should be reflected in a loss of nitrogen from control plants. In view of the fact that the figures given for seed nitrogen are only average values, and that individual seeds, even of the same weight, vary in their nitrogen content, the number of control pots in the present experiments is too small to permit of a clear indication being given of any tendency to slight loss of plant nitrogen. In the earlier investigation (Bond, 1936, p. 569) it was noted that the general tendency was for a loss to occur. It is quite possible that the higher figure obtained in experiment IV (see above) may have been due to a further process of actual decay of roots, as the result of the constricted rooting conditions within the small pots used in this particular experiment. On the other hand, the failure of Virtanen, von Hausen, and Laine (1937) to detect any similar increase in sand nitrogen in control pots of peas under strictly aseptic conditions lends some support to possibility (b) above, although it should be noted that the duration of these experiments with soya bean was considerably greater than with those of Virtanen *et al.* with pea, giving more time for sloughing-off or root decay to occur.

In connexion with the possible activity of non-symbiotic nitrogen fixing organisms in circumstances such as those of the experiments described in this paper, the following trials were devised with the object of ascertaining whether such activity was appreciable in pots containing plants similar in size and vigour to nodulated plants. Two uninoculated pots from experiment I were supplied with two doses of nitrate, one at the commencement and the other midway through the experiment, well-developed plants being thus obtained. Nitrogen added and recovered were as follows:

N added to each pot:	In seeds	.	.	.	.	37.5 mg.	} 120.0 mg.
	As NaNO ₃	.	.	.	.	82.5 "	
N recovered after three months' growth:							
						Pot 1.	Pot 2.
	In plants	.	.	.	.	122.0 mg.	117.0 mg.
	In sand	.	.	.	.	8.0 "	9.0 "
	Total recovery	.	.	.	.	130.0 "	126.0 "

When it is recalled that these plants received several milligrammes of nitrogen from the water-supply (see Tables I and II) it becomes clear that any non-symbiotic fixation was negligible.

V. SUMMARY

Experiments are described which were designed to detect any leakage or excretion of nitrogenous substances from nodules of soya bean plants (var. 'Manchu') growing in sand culture. The cultures were open to the atmosphere and are not to be described as sterile, though all materials were initially sterilized. Three different strains of the root-nodule bacillus were employed.

Nitrogen estimations were performed on sand in which nodulated soya beans had been grown, the sand from a total of thirty-three pots being examined. A slight increase in sand nitrogen was detected, amounting to a few milligrammes per pot, but the increase did not exceed, or exceeded by a trifling amount, the increase obtained in the sand from control pots with non-nodulated plants. There was, therefore, no evidence of the occurrence of appreciable excretion.

This result was confirmed in other experiments in which barley plants were grown in the same pots as soya beans. There was no evidence whatever of uptake of nitrogen by the barley plants.

After giving due consideration to the possibility of immediate reabsorption of the excretion having occurred in the experiments depending on sand analysis, and to that of non-availability of the excretion in the mixed cultures, it is concluded that in every probability there was no appreciable excretion in these experiments.

It is impossible to say at present whether this lack of excretion arises from (a) the absence of excretion from soya-bean nodules at any time, or (b) the absence from the present experiments of some so far unidentified condition necessary for excretion to proceed. It is at least clear that excretion is not an invariable accompaniment of fixation of nitrogen within leguminous nodules.

The bearing of these experiments upon a previous investigation by the author is considered.

In addition to acknowledgements already made, the author expresses his indebtedness to Dr. S. Williams, who made certain suggestions in connexion with the manuscript, and thanks the Carnegie Trustees for a grant in aid of publication. Mr. I. W. Prentice gave valuable assistance with the 1936 experiments.

ADDENDUM

Virtanen (Nature, cxl. 683. 1937) has suggested that size of sand-particle may be a decisive factor in excretion. The use of sieves shows that 80 per cent. by weight of the sand used in the experiments reported in the present paper is composed of particles with diameters within the range 0.1 mm. to 0.9 mm., 10 per cent. consisting of particles below and 10 per cent. of particles above this range. A sieve of 0.5 mm. mesh transmits 50 per cent. by weight of the sand. In terms of number of particles it is clear that the diameters of the majority of particles will be in the lower part of the range mentioned. Very similar sand was used in most of the writer's previous experiments on transfer of nitrogen.

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Morphological and Cytological Studies in the Capparidaceae

II. Floral Morphology and Cytology of *Gynandropsis pentaphylla* DC

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With Plate I and fifty-seven Figures in the Text

INTRODUCTION

INFORMATION available on the cytology of the members of the Capparidaceae is very meagre. The chromosome numbers that have been determined in the family are almost exclusively confined to a few species of only two genera, namely, *Cleome* and *Capparis*. The family is almost entirely tropical in its distribution, and in India it is represented by about eight genera. Tischler (1921) refers to *Cleome paradoxa* in his treatise on the significance of chromosomes in heredity. Taylor (1925) mentions the diploid chromosome number of *Cleome spinosa* as thirty-eight in his paper on chromosome morphology as determined by the position of attachment constrictions. A comparative treatment of *Cleome spinosa* and *C. gigantea* is made in Ufer's (1927) paper. Schiller (1928) emphasized the presence of 'Dauerchromosomen' in *Capparis spinosa* and recorded the presence of transverse division of the chromosomes at the end of somatic prophase. This was, however, contradicted by Kuhn (1928) who, working on the same species, found that there was present only normal longitudinal division of the chromosomes. There was also disparity in the chromosome number reported by the two authors. Tischler (1931) says that 'die von Schiller angegebene Zahl 12 ist wohl unrichtig'. Since this paper was being prepared for the press I have seen a paper by Rao (1937) on the embryology of *Gynandropsis pentaphylla* in which he records the chromosome number as $n = 16$, whereas I find it to be seventeen. The plant under investigation is a weed on waste lands in the drier parts of south India and occurs in association with *Cleome viscosa*, which it closely resembles.

MATERIALS AND METHODS

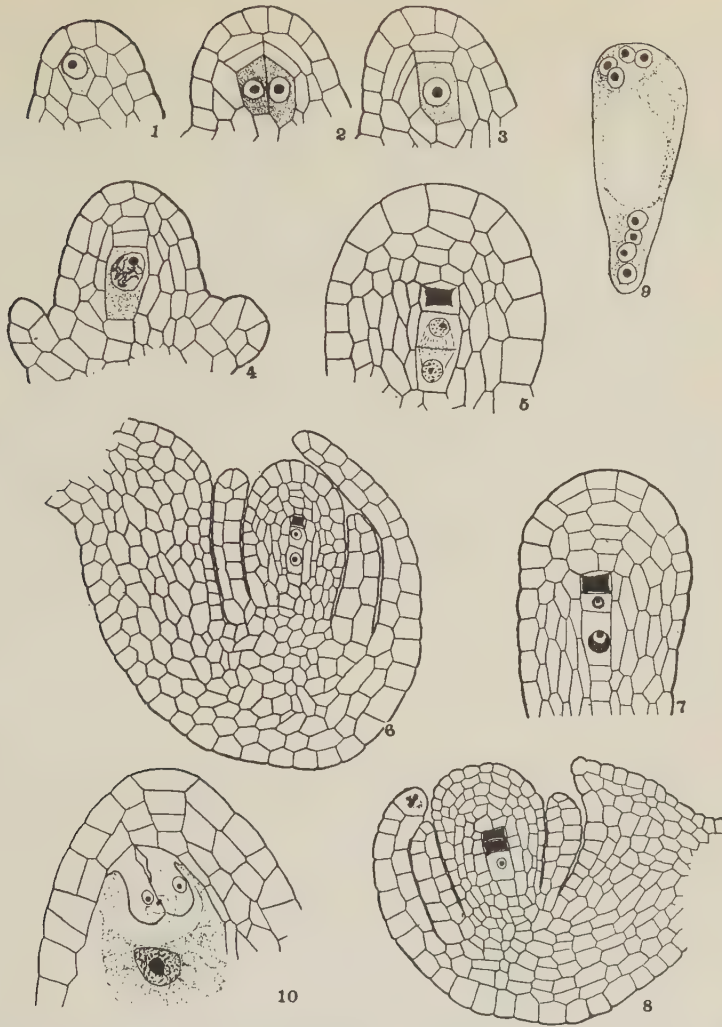
Plants of *Gynandropsis* were raised from seeds got from Madras, south India, and South Africa, in the greenhouse at the Genetical Laboratory, Regent's Park Botanical Gardens. The chromosome behaviour of the three is so similar that there is no need to treat them separately. The flower buds of

the required stage, after removal of the perianth, were fixed in various osmic fixatives. A prefixation in Carnoy or absolute alcohol was found quite essential for facilitating the sinking of the buds. By far the best results were obtained with Benda's fluid. Next in order came 2 BE (La Cour, 1931) and then chrom-acetic formalin of Karpechenko and Langlet (Manton, 1932). The determination of the right stage by aceto-carmin examination offered some difficulty owing to the anthers of a flower being in different stages of development, so that if one came across first metaphase in aceto-carmin the sections revealed tetrads and diakinesis. Sections which were cut at a thickness ranging from six to twelve microns were stained in iodine gentian violet and in iron-alum-haematoxylin.

For stages later than diplotene, smears were found to be more useful than sections. Benda gave very satisfactory fixation results. In addition uranium acetate ($2\frac{1}{2}$ per cent. aqueous solution) was tried with considerable success. Fixation for an hour was found to be sufficient, and no bleaching is necessary. Further experimentation with what promises to be a most useful fixative, by addition of varying quantities of acetic acid and perhaps saponin, may enhance its value to the level of good osmic fixatives. Smear preparations were invariably stained with iodine gentian violet. The procedure that was found necessary to ensure proper staining was the one involving the use of chromic acid as mordant after gentian violet.

The origin and development of the megaspore.

This is closely parallel to the details already described for *Cleome chelidonii* (Raghavan, 1937). The hypodermal archesporium may be either one cell (Text-fig. 1) or frequently two (Text-fig. 2). A large amount of parietal tissue is formed before the megaspore mother-cell functions, by the activity of the primary parietal which is derived from the periclinal division of the archesporial cell (Text-figs. 3 and 4). The heterotypic division results in two daughter-cells, of which the one towards the micropylar end does not function any further and degenerates (Text-fig. 5). The lower one divides again and forms two megaspores, of which the lower enlarges and functions as the embryo-sac. Thus there is a linear triad rather than the usual tetrad. Text-fig. 5 shows the uppermost cell degenerated and the lower in the process of division, while Text-figs. 6 and 7 indicate a later stage, wall-formation between the two lower cells having been completed. Text-fig 8 represents a still later stage, in which there is a linear triad with the upper two degenerated and the lowest functioning. Schnarf (1936), in reviewing the various types of sporogenesis and selection of the functioning macros pore belongs the degeneration, after the first and before the second division, of one of the two daughter-cells, the one nearer the chalazal end. In this case, however, it is the one nearer the micropylar end that degenerates. Coulter (1908) thinks that the number of divisions from the embryo-sac mother-cell to the egg



TEXT-FIGS. 1-10. Fig. 1. Single archesporial cell of the ovule. $\times 600$. Fig. 2. Two juxtaposed megaspore mother-cells with two layers of wall-cells. $\times 600$. Fig. 3. Single megaspore mother-cell. The primary parietal cell has undergone tangential septation. $\times 600$. Fig. 4. Enlarged megaspore mother-cell with about four layers of wall-cells surrounding it. Note the origin of the integuments. The nucleus is in early zygotene. $\times 600$. Fig. 5. The cell towards the micropylar end resulting from the heterotypic division of the megaspore mother-cell has disintegrated. The lower one is in a process of division. $\times 600$. Fig. 6. The wall between the two cells is complete. Linear triad with the topmost cell disintegrated. Note the outer integument overtaking the inner, though it is initiated slightly later. Note also the heavy parietal tissue in which the triad is embedded. $\times 400$. Fig. 7. The lowest of the triad enlarging, and the middle one is in a process of disintegration. $\times 600$. Fig. 8. The two upper cells disintegrated. The lowest and functioning megaspore enlarging. $\times 400$. Fig. 9. Eight-nucleate embryo-sac. The antipodal nuclei arranged in a vertical row. $\times 800$. Fig. 10. The synergids showing the 'Hakenförmige Leistenbildung'. The polar nuclei have fused and taken up a position near the egg-apparatus. $\times 800$.

furnishes the most valuable data for phylogenetical and systematic studies. A case like this, where there are only four divisions instead of the usual five between the embryo-sac mother-cell and the eight-nucleate embryo-sac, will have to be considered, on this basis, as a more advanced type. Ernst (1908), rejecting Coulter's view, recognizes two distinct processes in the life-history of the gametophyte: embryo-sac formation and embryo-sac development. The latter process is determined by the number of divisions, by the arrangement of the nuclei and vacuolization. He instances the Liliaceae, where all types of megaspore formation are found, from the normal tetrad down to total suppression. Always, however, the functioning megaspore, by three divisions, reaches the eight-nucleate state. Ernst thus disagrees with Coulter's concept that the number of divisions by which the eight-nucleate sac is reached will serve as a criterion of homology. The divergence in this matter between two closely related genera as *Cleome* and *Gynandropsis* rules out, in my opinion, any possibility of phylogenetic significance being attached to the number of divisions between the megaspore mother-cell and the eight-nucleate sac. The synergids have the 'Hakenförmige Leistenbildung' as in the case of *Cleome* (Text-fig. 10), and the behaviour of the egg and the zygote is the same. In a particular eight-nucleate embryo-sac prior to the migration of the polar nuclei (Text-fig. 9) the four antipodal nuclei were arranged one below the other, a rather unusual form of disposition.

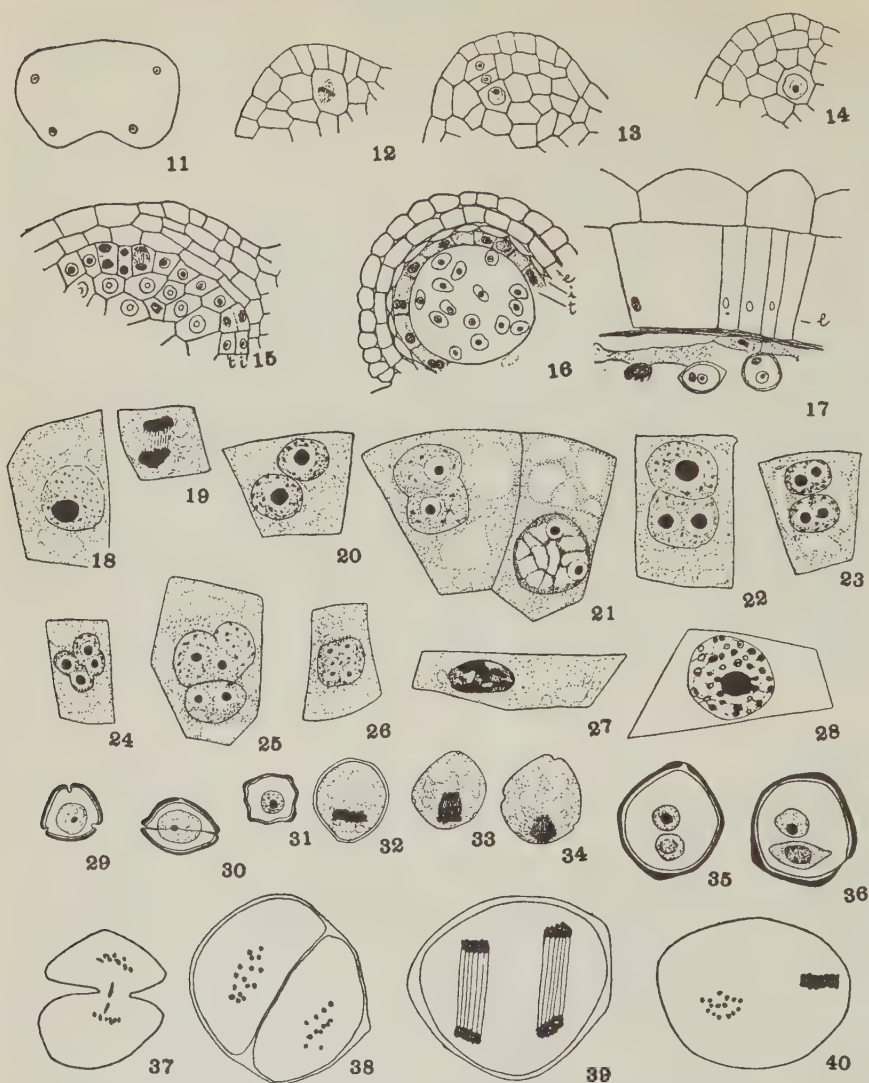
The origin and behaviour of the tapetum.

The archesporium of the anther differentiates as four hypoderms, one below each corner of the anther in which the lobing is scarcely evident at this stage (Text-fig. 11). It cuts out a parietal cell (Text-fig. 12) which, as in the case of the megaspore mother-cell, is more active in the first instance than the microspore mother-cell. It immediately divides (Text-fig. 13) periclinally, followed by similar divisions of adjacent cells (Text-fig. 14). By repeated divisions four layers of wall-cells are formed, of which the innermost functions as the tapetum. Text-fig. 15 shows the cells of the innermost wall layer in different stages of division; the ones immediately adjacent to the pollen mother-cells are the tapetal cells (*t*) whose nuclei are more prominent than in the rest of the wall-cells. The intermediate layer of cells (Text-figs. 15 *i* and 16 *i*) cut off from the tapetum, caught as it were between the enlarging tapetal cells and the endothelial cells (Text-fig. 16 *e*), gets tangentially stretched and ultimately crushed. In a fully differentiated anther one finds the pollen mother-cells closely packed and the surrounding tapetum forms an intrusive layer. Such an inpushing of the tapetal cells into the microsporangial cavity has been reported by Gregory (1936) in *Elettaria cardomomum*. In fact the tapetum is so very much adpressed to the sporogenous tissue and so like it in appearance that one not tracing its ontogeny may easily mistake it as being derived from the sporogenous tissue. It was this, perhaps, that led Coulter (1898) to observe in *Ranunculus* that in some cases the whole

of the tapetum seemed to be cut off from the periphery of the sporogenous tissue. The hypodermal layer (Text-figs. 16 and 17 e) then enlarges, shows great radial elongation, and functions as the endothecium.

The tapetal cell, to begin with, consists of a comparatively prominent nucleus and cytoplasm with large vacuoles (Text-fig. 18). The nucleolus is very large and small irregular bodies, obviously the prochromosomes, are distributed peripherally around the nucleolus. At the time the microspore mother-cells are about to enter upon the meiotic stages the tapetal cells become binucleate. Text-fig. 19 shows the nucleus of the tapetal cell in the telophase of division, which disproves any possibility of an amitotic division by which the tapetal nuclei are held by some authors to multiply. Text-fig. 20 shows the binucleate tapetal cell. Thereafter the behaviour of the tapetal nuclei is rather irregular. Sometimes these two nuclei fuse and form a single large binucleolated nucleus. Text-fig. 21 shows two adjoining cells, in one of which the two nuclei are in a process of fusion while the other shows the fused product. The most common behaviour is for the two nuclei to divide again, the resulting four nuclei fusing immediately. In fact this fusion follows so quickly upon the division that it is hard to find them in a separate condition. Text-figs. 22-6 show them in various processes of fusion. A nucleus is organized ultimately in which can be seen the four nucleoli of the fused nuclei (Text-fig. 26). Not infrequently all these stages are represented side by side in the same tapetum. Gates and Rees (1921) have reported that in *Lactuca* the tapetal cells are quadrinucleate on one side of the loculus and binucleate on the other.

The chromatin bodies in the meantime become coarser and denser and the nucleoli becoming merged with these, the nucleus at pollen-grain stage presents an ovoid appearance with its contents disintegrating (Text-fig. 27). The cell itself is greatly stretched tangentially and ultimately torn. The contents of the cells and nuclei pour into the sporangial cavity and are presumably utilized by the developing pollen grains. Cooper (1933), after investigating forty-three species of angiosperms, groups them into three classes according to the behaviour of the tapetal nuclei: (i) in which the mature tapetal cell is uninucleate, (ii) in which the nucleus divides once and is binucleate, and (iii) plurinucleate tapetal cells. But he thinks that the plurinucleate condition arises from failure to complete the nuclear divisions. The observations herein recorded show that the nucleus divides first and later there is a definite fusion. Recently Bhargava (1936) and Singh (1936) have recorded a similar fusion of nuclei in the tapetal cells of *Chenopodium* and *Ranunculus* respectively. Bonnet (1912), who has made an elaborate study of the problem, observes: 'Parallèlement aux phénomènes de multiplication nucléaire qui tendent à rendre la cellule polyénergide, se produit un processus inverse, qui tend à centrer à nouveau la cellule autour d'un noyau unique. . . . Ces fusions peuvent s'effectuer entre deux ou plus de deux noyaux.' This is in agreement with the observations herein recorded.



TEXT-FIGS. 11-40. Fig. 11. The origin of the archesporium of the anther as four hypoderms below each corner of the anther sac. $\times 240$. Fig. 12. The archesporial cell dividing to cut off the primary parietal cell. $\times 350$. Fig. 13. The primary cell has undergone periclinal division. $\times 350$. Fig. 14. The undivided microspore mother-cell is surrounded by three layers of wall-cells. $\times 350$. Fig. 15. Tapetum (*t*) being derived from the division of the innermost wall layer (*i*). $\times 350$. Fig. 16. A mature anther-sac. *e* = endothecium, *i* = intermediate layer, and *t* = tapetum. $\times 240$. Fig. 17. The endothecial cells (*e*) greatly elongated radially. The intermediate layer crushed and the tapetal cells disorganized and their contents flowing into the cavity of the anther-sac. $\times 350$. Fig. 18. Uninucleate tapetal cell. Note the highly vacuolated nature of the cytoplasm. $\times 800$. Fig. 19. Nucleus of the tapetal cell in telophase of division. $\times 800$. Fig. 20. Binucleate tapetal cell. $\times 800$. Fig. 21. Two adjoining cells of the tapetum. In the one on the left the two nuclei are just fusing, while in the other

The microspore.

Text-figs. 29-34 show pollen grains in various views and different stages of germination. The exine is thick, and at some regions is thicker than in others. The nucleus to begin with is centrally situated and enlarges greatly prior to division (Text-fig. 30). It then migrates to a peripheral position and in Text-figs. 32 and 33 are seen the metaphase plate in side view as also telophase. The two nuclei resulting from the division are at first equal in size, but the generative cell ultimately assumes a lenticular shape (Text-fig. 36). At telophase, represented in Text-fig. 34, a clear plate across the spindle could be seen, which cuts the microspore into two cells of very unequal size. The one towards the microspore wall is the generative cell. It rounds itself off from the wall of the pollen grain and organizes itself into a lenticular cell which, as it were, comes to be embedded in the cytoplasm of the pollen grain. It is at this stage that the microspores are shed.

Microsporogenesis.

The pollen mother-cells occur as a closely packed cylindrical column pressed against the tapetum and consisting of several longitudinal rows. Each pollen mother-cell is irregularly polygonal in outline and they fit closely together. They lose their angularity and separate from one another at about diakinesis. A discussion of the early stages of meiosis and the prochromosomes in Gynandropsis and the related genus Polanisia is given in a separate paper.

Diakinesis.

While at diplotene the paired threads more or less crowd around the nucleolus, at early diakinesis the thirty-four chromosomes, which form invariably seventeen pairs, are approximately equidistant from one another (Pl. I, Fig. 2), around the periphery of the nucleus. This, according to Lawrence (1931), is due to a repulsion phase which begins at earliest prophase and continues till mid-diakinesis, by which time the chromosomes are sharply defined. The spacing of the seventeen pairs is fairly regular, and, moreover, the members of each pair are seen to be repelled to about the same distance from one another. Gates (1909) first observed a uniform spacing of the bivalents in *Oenothera* at diakinesis, and suggested even then that this was due to a mutual repulsion of the bivalents.

In over thirty nuclei examined at diakinesis, no case was found suggesting

they have fused. $\times 800$. Figs. 22-6. The nuclei in various stages of fusion. $\times 500$. Fig. 27. A disintegrating tapetal cell. $\times 800$. Fig. 28. Archeporial cell of the ovule. Thirty-four prochromosomes are seen with four attached to the nucleolus. $\times 1,500$. Figs. 29-34. Pollen grains in various views. The nucleus in various stages of division. $\times 800$. Fig. 35. Mature pollen grain in sectional view; the lower one is the generative cell. $\times 800$. Fig. 36. The lenticular shape of the generative cell is shown. $\times 800$. Figs. 37, 38. Two abnormal cases of cytokinesis. $\times 1,500$. Fig. 39. The homotypic spindles in the same plane. $\times 2,500$. Fig. 40. The homotypic spindles at right angles to one another. $\times 2,500$.

association of more than two chromosomes. Multivalent formation can therefore be said to be absent at this stage.

The bivalents are of two kinds, excluding the two lagging ones of which mention will be made later. In the first type the members are connected at one end only, forming a rod-shaped pair. In the second, there are connexions at both ends, giving a ring bivalent (Pl. I, Fig. 2R). Lawrence (1931) observes that the first type, which is characteristic of all small chromosomes, arises out of a swinging apart and subsequent orientation of the component members, so that the two chromosomes are apparently paired end to end. Nandi (1936), who has made an analysis of the chromosome complement of *Oryza*, finds the smallest chromosomes forming one chiasma in one arm, and terminalization of this results in a rod bivalent. The longer bivalents form ring bivalents. In this case two ring bivalents are recognized in diakinesis and first metaphase (Pl. I, Fig. 2, and Text-fig. 53). This is borne out by four chromosomes of the somatic complement being definitely longer than the rest (Text-fig. 56). Obviously it is these that form the ring bivalents with two terminal chiasmata in each. From mid-diakinesis onwards the repulsive force gradually diminishes and the bivalents move away from the periphery toward the centre of the nucleus (Pl. I, Fig. 4). Side by side with this the members of each pair come close together and the previously attenuated connexions shorten and thicken. In Gynandropsis the chromosomes evidently lose their charge and aggregate in the centre of the nucleus before the nuclear membrane disappears.

Prometaphase.

At the close of diakinesis this converging movement becomes accentuated, resulting in the crowding of the chromosome pairs in the centre of the nucleus. Thus the transformation from early diakinesis to prometaphase is marked by a migration of the bivalents from the periphery to the centre of the nucleus and a close approximation of the chromosomes of each bivalent to such an extent that their duality becomes almost obscured. Prometaphase is characterized by yet another phenomenon, that of the grouping in a definite manner of the bivalents which hitherto remained separate from one another, distributed equally either near the periphery or the centre of the nucleus. Pl. I, Fig. 5, shows prometaphase bivalents in four groups of two, one of three, and six singles. This is the beginning of the secondary association of chromosomes which manifests itself in a prominent manner in the succeeding metaphase and anaphase, and of which a detailed description is given below. The bivalents undergo orientation in such a manner that they come to lie in the same direction as the spindle fibres.

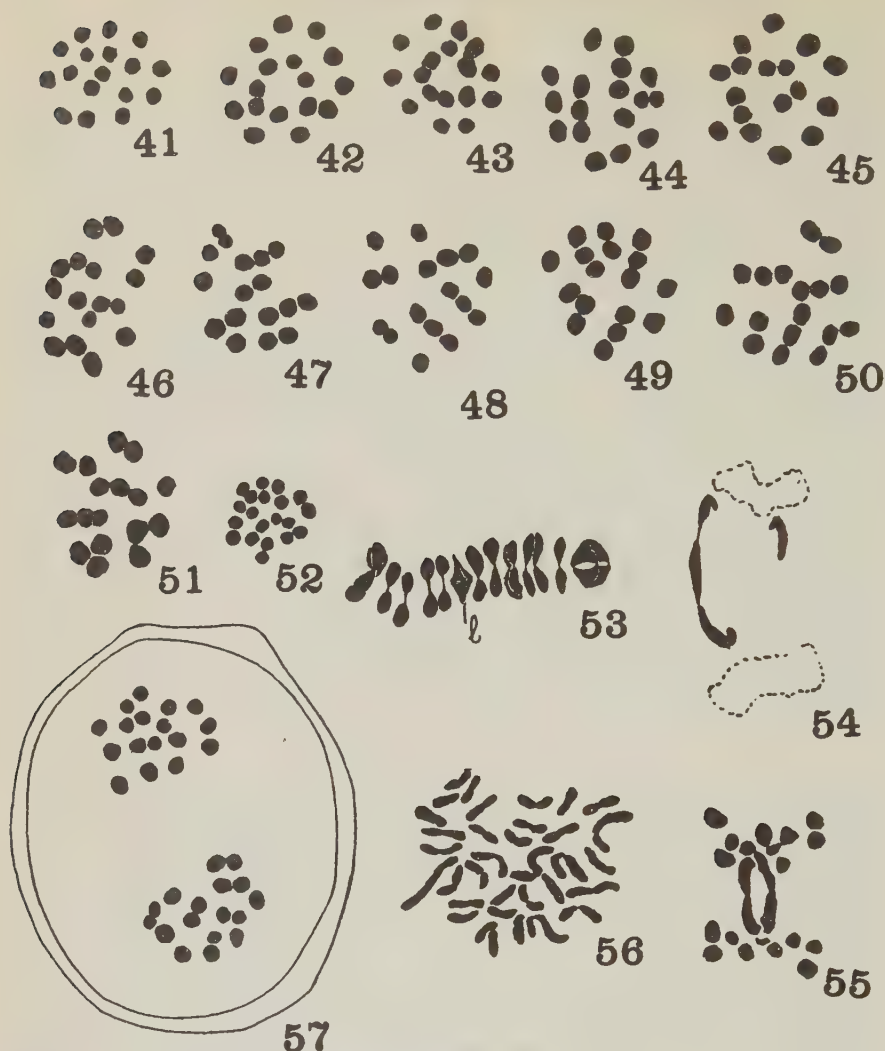
Metaphase.

Prometaphase is succeeded by metaphase, the most characteristic feature of which is the approximation of the separate bivalents. In well-fixed preparations this association never tends to actual contact. This secondary

association is so very prevalent that very few metaphase plates were found without secondary pairing.

Kuwada (1929) compares the chromosome arrangement with that of Mayer's (1879) floating magnets. Mayer has shown the configurations formed by numbers of magnets extending from two to twenty. The number of needles forming the configuration was floated in a bowl of water. The eye ends of the needles, which protruded a short distance beyond the tops of corks, were of S-polarity. A cylindrical magnet, 30 cm. long and 5 mm. in diameter, was clamped in a vertical position with its N-end at the constant distance of 60 mm. above the tip of the needle which floated in the line of axis of the magnet. The configurations made by the floating magnets fall into well-marked groups or classes—primary, secondary, tertiary, quaternary, &c. The stable configuration of one class forms the nucleus to the succeeding ones. That configuration is the most stable which has the least number of needles for its nucleus. The stable configuration of seventeen, which comes within the secondary group, consists of a 'nucleus' of six plus one, with ten arranged in a ring around it. The stable primaries form the nuclei of the secondaries. An examination of a large number of nuclear plates revealed that the stable form of arrangement conforms to this type, but this is distorted to a considerable extent by secondary association. Text-figs. 41–51 show a number of plates arranged according to the number of associations exhibited by the component chromosomes and starting from Text-fig. 41, which shows only one association and in which could be seen the stable configuration 10 (6+1), one can see how this arrangement is gradually distorted or interfered with as the number of associations increases. Alam (1936) has found a similar distortion of the theoretical arrangement of the metaphase chromosomes in the Cruciferae due to the effect of secondary pairing. Nandi (1936), however, considers that the mechanism of chromosome distribution on the metaphase plate is not disturbed by secondary association and that when secondary association is present the groups of paired bivalents act as single bodies and arrange themselves in accordance with a corresponding number of floating magnets. He attributes the distorted appearance of the type described here to the fact of the chromosomes being fixed at various stages of the oscillating movement which characterizes the chromosomes at diakinesis, the chromosomes acting as floating bodies which mutually repel each other. Whatever the cause, I have not been able to see anything but a distortion brought about by the secondary association. In *Capparis sepiaria* (Text-fig. 52) the twenty bivalents are all in pairs. This is very similar to the condition observed by Hagerup (1931) in *C. Rothii*, and he says: 'Die Chromosomen liegen deutlich paarweise angeordnet (Text-fig. 8), was bedeuten kann, dass die Art tetraploid ist.'

Analysis of side-views of all metaphase chromosomes is rendered difficult not only because of the large number but because of secondary association, and the secondarily paired chromosome groups often lie one below the other



TEXT-FIGS. 41-57. Figs. 41-51. *Gynandropsis* pollen mother-cell nuclei showing varying degrees of secondary associations. In Fig. 41, with only one group of two, the stable form of magnetic arrangement 10 (6 + 1) is seen. Note how this is distorted as the number of associations increases. Figs. 50 and 51 show the maximum association. $\times 5,000$. Fig. 52. *Capparis sepiaria* pollen mother-cell; Metaphase I from aceto-carmin preparation ($n = 20$), all the bivalents in pairs. $\times 3,000$. Fig. 53. Side-view first metaphase, *Gynandropsis*. 1 = a group of two bivalents with incompletely terminalized chiasmata. $\times 5,000$. Figs. 54, 55. Lagging bivalents. First anaphase, *Gynandropsis*. $\times 5,000$. Fig. 56. Metaphase polar view root-tip of *Gynandropsis* ($2n = 34$). $\times 5,000$. Fig. 57. Second metaphase, both plates in polar view. Note six groups of two in the lower plate. $\times 5,000$.

so that their perpendicular disposition with reference to the field of the microscope makes them difficult of resolution. In Text-fig. 53 all the seventeen bivalents are shown in side-view of first metaphase. They have been separated

laterally while drawing, care, however, being taken not to interfere with the bivalents that are secondarily paired. It is obvious that the paired bivalents of one group show similarity in size, configuration and in the number and position of the chiasmata. Six groups of two and five separate bivalents are shown in the Text-fig. 53. Of the six groups of two, one group is made up of ring bivalents possessing two terminal chiasmata. Two seem to possess incompletely terminalized chiasmata (Text-fig. 53 *l*). The rest belong to the rod-shaped type with one terminal chiasma each.

Secondary pairing.

A variable number of bivalents are seen to be secondarily associated. This pairing, which becomes evident at prometaphase, is exhibited very clearly in first metaphase and first anaphase, and less clearly in second metaphase (Text-fig. 57). Table I gives a summary of the various types of secondary

TABLE I
Types of Secondary Association

No. of sec. assns.	No. of bivalents in assn.			No. of cases.	Total.
	1.	2.	3.		
1	15	1	—	1	1
2	13	2	—	2	2
3	11	3	—	2	2
5	9	1	2	2	2
6	5	6	—	5	17
	6	4	1	6	
	7	2	2	6	
7	5	3	2	4	9
	4	5	1	5	
8	3	4	2	6	10
	4	2	3	4	
9	2	3	3	3	4
	3	1	4	1	
10	1	2	4	2	2
					—
					49

association. In computing the number of secondary associations in each case, an association of three bivalents is counted as two and that of two bivalents as one. The number of secondary associations per plate ranges from one to ten, the mode being six. The maximum of ten has been seen twice in the forty-nine plates examined (Text-figs. 50 and 51). It shows four groups of three, two groups of two, and a single, making seven separate associations.

Anaphase 1.

In anaphase secondary association is maintained to the same extent as in first metaphase. As to the forces that are responsible for the anaphasic separation, divergent views have been held. Kuwada (1932) assumes that this is due to polar attraction while Darlington (1932) insists on a polar repulsion which he considers essential for metaphasic equilibrium. Since it is a fact that

pieces of cork with magnetized needles representing the chromosomes take up the stable configuration only under the influence of a magnet that bears the charge opposite to that of the needles, it is evident that the poles tend to exert an attractive rather than a repulsive force, for a magnet of the same charge brings about a dislocation and scattering of the arrangement. Catcheside (1934) describes the attachment constrictions as being the seat of localized forces leading to a mutual repulsion of the chromosomes of a bivalent. This is derived from a release of the particulate attractions which led to the pairing of the chromatids at zygotene, brought about by the condensation of each chromatid at first metaphase. In common with Alam (1936), I believe that the anaphasic separation is brought about by both these forces, repulsion between attachment constrictions and the attractions of the poles.

Lagging bivalents are frequently met with and two particular bivalents always seem to lag. Their configuration (Text-figs. 54 and 55) at separation indicates that this is due to the presence of interstitial chiasmata. In Text-fig. 53, representing side-views of first metaphase, two bivalents (*l*) are seen in which there is suggestion of interstitial chiasmata, and it is likely that these are the ones that lag. This is in keeping with the observations of Catcheside (1934) on the Cruciferae.

Interkinesis.

After the chromosomes have reached the poles, each group organizes a telophase nucleus. The nucleolus makes its appearance and the chromosomes are more or less uniformly distributed as in diakinesis. Gates (1909) first recorded the uniform spacing of the chromosomes at interkinesis in *Oenothera*, and this he attributed to a mutual repulsion. The clumping of the chromosomes at early telophase is, according to him, due to attraction. But 'the medium in which bodies float frequently changes their qualities of attraction and repulsion, and it appears that the repulsion first develops after the appearance of the karyolymph in which the chromosomes float'. No partition wall is formed between the daughter nuclei (Pl. I, Fig. 6), nor is any resting condition of the nuclei found at interkinesis. The chromosomes, however, seem to be connected by thin strands. This appears to represent a stage analogous to early prophase described elsewhere, where the prochromosomes seen in the resting stage as irregular bodies, being portions of thin strands which form the 'reticulum', become more prominent at a later stage. This stage at interkinesis is similar to the one described above, so that it would appear that the seventeen bodies organized at this time are the prochromosomes and the thin lines connecting them are the chromatin threads. Further continuance of this process which is the reverse of the earlier resolution of the chromosomes from prochromosomes would, by the diminution of chromatin in these prochromosomes, result in the attainment of the resting condition. But since no resting condition is reached during interkinesis, it is at once followed by second metaphase.

Homotypic divisions.

The two unseparated daughter nuclei divide nearly simultaneously. The spindles may be in the same plane (Text-fig. 39) or at right angles to one another (Text-fig. 40). At second metaphase the chromosomes assume the arrangement of floating magnets, but secondary pairing, which persists even up to this stage, distorts the arrangement. Text-fig. 57 shows both the chromosome groups in polar view. The lower plate shows six groups of two and five distributed singly. At second telophase (Pl. I, Fig. 9) the distribution of the prochromosomes resembles markedly that found at interkinesis. The connecting fibres are also apparent, and as the nuclei pass into the resting condition synchronizing with the organization of the microspore, these chromatin bodies become paler and the strands become slightly more chromatic and present the appearance described for the pre-meiotic nuclei of the pollen mother-cell. The tetrads are of either the tetrahedral (Pl. I, Fig. 9) or the quadrite type (Pl. I, Fig. 10). It may be of interest to record here that several stages of meiotic divisions are represented side by side in the same anther lobe. It is quite common to see in the smear preparations of a bud, tetrads at some portions, first metaphase at some other, second anaphase at another, and so on.

Abnormalities of cytokinesis.

Though as a rule no cytokinesis takes place after the first division, a few mother-cells were seen in which the daughter nuclei had been separated by a wall (Text-fig. 38), so that two daughter-cells are rounded off while the chromosomes were in second metaphase. In another case (Text-fig. 37) the pollen mother-cell is seen undergoing a process of constriction (presumably in a process of division) while the chromosomes are in the telophase of the first division. Such and other abnormalities seem to be widely prevalent in the closely allied *Papaveraceae*, as disclosed by the observations of Yasui (1931).

The nucleolus; its organization and behaviour.

The nucleolus is organized as two distinct bodies in the telophase preceding interphase (Pl. I, Fig. 8). To each nucleolus is attached a prochromosome. This indicates that the origin of the two nucleoli is governed by two distinct meiotic chromosomes. Very often one of the two nucleoli is bigger than the other, perhaps due to competition. Heitz (1931 *a*) showed that when two satellited chromosomes were present in a monoploid complement, distinctive size differences of the nucleoli could result and that this could be correlated with the differential functioning activities of the two sat.-chromosomes. Both at the resting condition of the pollen mother-cell and the prophase of meiosis there is, as a rule, only one large nucleolus. This must obviously be due to fusion of the two nucleoli which must have originated as four bodies in the last telophase of the pre-meiotic division. I was not able to see this in the pre-meiotic nuclei, but an examination of the cells of the root-tips revealed that in

the telophase of somatic mitosis, the nucleoli originate as four irregular masses, to each of which a prochromosome is found attached (Pl. I, Fig. 7). A fusion such as the one suggested is also in agreement with the observations of Heitz (1931 *b*), who observed that fusion of such nucleoli could occur very early in the telophase. Pl. I, Fig. 1, shows the nuclei of the pollen mother-cell at zygotene. The paired threads are so delicate that it is impossible to count them at that stage. There are two deeply staining bodies attached to the nucleolus. These are obviously the nucleolar bodies in connexion with the two pairs of zygotene threads. Very often the paired threads are attached to the nucleolus with a bud of the nucleolus at the attachment region. Smith (1933) found a similar bud of the nucleolus in *Galtonia* and found that the two satellited chromosomes form a bivalent whose satellites are independently attached to the nucleolus. At all phases of diakinesis two bivalents occur attached to the nucleolus (Pl. I, Figs. 2 and 4). In Pl. I, Fig. 6, where there are two nucleoli, a bivalent is attached to each. At interphase two nucleoli occur, to each of which is attached a prochromosome (Pl. I, Fig. 8). There seems to be no doubt, therefore, that these nucleoli are organized by the chromosomes to which they are attached. This means that the somatic complement should possess four satellited chromosomes. I was not, however, able to see the satellites in the prophase or metaphase chromosomes. But the fact that the nucleoli originate as four separate bodies (Pl. I, Fig. 7) is a fair indication that two pairs of satellited chromosomes are responsible for their organization. This is also corroborated by four prochromosomes being attached to the nucleolus in the archesporial cell of the ovule (Text-fig. 28), prior to the cutting-off of the primary parietal cell.

DISCUSSION

Secondary association.

The association of bivalents at meiosis was first observed by Kuwada (1910) in *Oryza sativa*. He found this only in second metaphase. Ishikawa (1911) found secondary pairing also at second metaphase of *Dahlia variabilis*. Since then various authors have found the phenomenon in different genera. Darlington (1928) originally advocated the theory of secondary pairing in his studies in *Prunus*. It was put on a systematic basis by Lawrence (1931), who has discussed all the then known cases of secondary pairing.

Secondary association may be defined as pairing of bivalents which are phylogenetically related, though in a distant way. It results from an attraction between such chromosomes; in other words, ancestral homology results in pairing. It is therefore 'intimately connected with allopolyploidy' (Lawrence, 1931). In typical allopolyploids derived from a previous cross there is a comparatively low proportion of multivalent formation. This means that the corresponding chromosomes derived from the two parents are not completely identical but are slightly different, which fact is manifested by the degree of attraction existing between the bivalents of the two parents. Usually, there-

fore, pairing takes place between chromosomes of the same parent, and this implies that syndiploidy has already been brought about, either by somatic doubling or by the fusion of unreduced gametes. Cases are, however, known which exhibit gradation from almost complete identity of the homologues derived from the opposite parents to strong difference between them. Poole (1931) showed that in the diploid hybrid of *Crepis rubra* $\times$ *C. foetida* there was complete pairing of the chromosomes, suggesting that they behaved as though they were from the same parents. The tetraploid derived from it behaved almost like an autotetraploid, quadrivalent formation being very common. On the other hand, Karpechenko (1927) has found that in the diploid hybrid, *Raphanus sativus* $\times$ *Brassica oleracea*, no pairing occurs, while in the tetraploid there are no quadrivalents. It is in cases such as this that secondary association helps in the determination of chromosome homology. The homologues from the two parents are different enough not to allow of pairing, but their affinity could always be inferred by the exhibition of secondary pairing between them. In other words, secondary association is a measure of the degree of affinity between the homologous chromosomes of the different parents.

Since the degree of secondary association is a measure of chromosome homology, according to Müntzing (1933) it is therefore a rough indication of the age of the species. He considers the so-called diploid varieties of potatoes to be really ancient allotetraploids. No multivalent association occurs in them, and therefore they are not of recent origin. Differentiation of the corresponding chromosome sets has proceeded far enough to prevent pairing between them. On the other hand, the high frequency of secondary association indicates, according to him, 'that the potatoes with 24 chromosomes are not very ancient allotetraploids for, if they were, differentiation would have been much more complete and little or no secondary association evident'. This course of argument suggests that an allotetraploid showing a high frequency of multivalent formation is to be considered a recent form inasmuch as differentiation has not proceeded far enough to inhibit pairing between homologues derived from opposite parents. This is an argument that may not be quite acceptable, because even in many experimentally produced allopolyploids there are cases where no multivalent formation is seen, though this depends mainly on how different the parents are before the cross. This cannot imply an ancient origin for them.

The case of *Primula kewensis* (Newton and Pellew, 1929) comes between the two described above. In their diploid form, *floribunda* chromosomes pair with *verticillata* chromosomes, thus resembling the *Crepis rubra* $\times$ *C. foetida* hybrid, but in the tetraploid no multivalent formation is seen and pairing takes place only between *verticillata* and *verticillata* and *floribunda* and *floribunda*. Thus it is abundantly clear that in most cases syndiploidy is a necessary antecedent of fertile allopolyploidy.

In Gynandropsis we find that the maximum number of associations, seen twice, gives seven groups of chromosomes; four groups of three, two of two,

and one bivalent separate. This means that the diploid set of thirty-four chromosomes belong to seven types, four of which are represented six times, two four times, and one twice. The haploid chromosome set may be represented by

AAA
BBB
CCC
DDD
EE
FF
G

Thus the original ancestor of Gynandropsis would have had a basic number of seven and *G. pentaphylla* is to be considered a secondary polyploid. The derivation of the chromosome complement $2n = 34$ from $b = 7$ (Gates, 1935) may be explained as follows. Let us designate the original ancestral form having a haploid set of seven chromosomes as A. Another type 'A' having the same chromosome number may well be imagined to arise from the original form by gene mutation or structural changes such as translocation, inversion, &c. The diploid hybrid between the two will be almost sterile owing to lack of pairing (compare Goodspeed and Clausen, 1927, in *Nicotiana*; Karpechenko, 1928, in *Raphanus* $\times$ *Brassica*). By amphidiploidy, a tetraploid plant with twenty-eight chromosomes is derived. Non-disjunction may lead to plants with additional chromosomes and by competition a thirty-four chromosome organism may survive. Or in the alternative, a cross between a tetraploid and a hexaploid is likely to give a stable plant with thirty-four chromosomes. Future genetical work may, in my opinion, confirm the polyploid nature of this species.

It is interesting to note that in *Brassica*, belonging to a closely related family, the occurrence of duplicate factors is well established (Pease, 1926; Kristofferson, 1924). For instance, the 'bulbing' in kohlrabi is governed by duplicate factors, so also its purple pigment, there being a 9 : 7 ratio of purples to greens in F_2 as a result of a cross with green savoy. Similarly two factors, K_1 and K_2 , were found to control the curly foliage of kale (Pease, 1926) and of broccoli (Kristofferson, 1924) as against the non-curling of the cabbage. In a similar manner the genetics of any character in Gynandropsis may show that it is controlled by duplicate factors, suggesting the polyploid nature of the plant.

In the Pomoideae, Darlington and Moffett (1930) have shown that the somatic number thirty-four is derived from a basic number seven. But the gametic constitution of the two types is different in that whereas here it is quadruply hexasomic, the Pomoideae are trebly so.

The chromosome numbers so far known in the Capparidaceae are given in Table II. They are confined exclusively to a few species of two genera, *Cleome* and *Capparis*, and the numbers range from $2n = 8$ to 140. I have tried to see if any light could be thrown on the phylogeny of the two groups, Cleo-

moideae and the Capparidoideae, on the basis of the chromosome numbers and the now suggested basic number for an important member of the Cleomoideae. But the numbers available are so few that they do not lend themselves to any interpretation along this line. It seems to me that all these are

TABLE II

Chromosome Numbers in the Capparidaceae (Gaiser, 1926, 1930, and Tischler, 1931)

	<i>n.</i>	<i>2n.</i>	
<i>Cleome gigantea</i>	—	70	Ufer (1927).
" " var. <i>gigas</i>	70	—	" (Tischler, 1931).
<i>C. paradoxa</i>	16	—	Tischler (1921-2).
<i>C. spinosa</i>	—	38	Ufer (1927).
* <i>C. Chelidonii</i>	10	—	Author.
* <i>Polanisia trachysperma</i>	—	20	"
<i>Cleome viscosa</i>	10	—	Janaki ammal (1933).
* <i>Gynandropsis pentaphylla</i>	17	34	Author.
<i>Capparis acutifolia</i>	—	ca 85	Kuhn (1928).
<i>C. Cynophallophora</i>	—	18	"
<i>C. saligna</i>	—	30	"
* <i>C. sepiaria</i>	20	—	Author.
<i>C. Rothii</i> Oliv.	20	—	Hagerup (1931).

Those marked * have been determined by me.

secondarily balanced numbers and very likely represent different balances of the same primary basic number seven. It is quite conceivable that from this primary basic number there arose first the Capparidoideae by allopolyploidy and then the Cleomoideae. This is in keeping with the fact that the Cleomoideae are exclusively herbaceous, and the Capparidoideae woody shrubs. Chromosome studies of more members of the family, which I hope to continue, may throw more light on the suggestions that have tentatively been made here.

The nucleolus.

The nucleolus has been the subject of much discussion. Navashin (1912) first discovered a pair of satellited chromosomes in *Galtonia*, and since he found the satellites attached to the surface of the nucleolus, he termed them 'nucleosomes'. He was led to conclude, from studies of somatic prophase, that these satellites were picked up by fine strands sent out by two chromosomes which thus became the satellite chromosomes. Though workers like Kuhn (1928), Metz (1927), and others observed the attachment of particular chromosomes to the nucleolus, the true significance was not well understood till Heitz (1931 *b*), demonstrated that the nucleolus originated in the telophase at the position of the satellite stalk or secondary constriction and that the number of nucleoli formed depended upon the number of satellite chromosomes present in the complement. These nucleoli fuse to form one if the members of the pair were near enough together to bring the nucleoli into contact. In

another paper (Heitz, 1931 *a*) he examined thirty-three species of *Vicia*, finding two or four satellited chromosomes normally present in each. He concluded that all plants probably have satellited chromosomes which give rise to the nucleoli in telophase. McClintock (1934) studied in detail a case of reciprocal translocation between chromosomes VI and IX in *Zea mays*, and showed that the nucleolus originated not from the stalk or secondary constriction but from an organized body in the chromosome directly adjacent to the stalk of the satellite. This deeply staining body, at the point where the satellite chromosome is attached to the nucleolus, she called the nucleolar-organizing body. From other observations she concluded that the number of nucleoli in somatic telophases is correlated with the number of satellite chromosomes, haploid cells having one, diploids two, and triploids three. Gates (1937) has recently given a review of the work done so far in the elucidation of the relation between the nucleolus and the chromosomes.

In the plant under investigation, satellite chromosomes could not be made out in the somatic prophase or metaphase, probably owing to the smallness of the bodies and their delicate nature. In meiosis, the two darkly staining bodies attached to the nucleolus represent undoubtedly either the satellites themselves or the nucleolar bodies which produce the nucleolus at the base of the connecting thread in the satellite chromosomes. Whatever the nature of the bodies, it is abundantly evident that there occur in the monoploid complement of *Gynandropsis* two satellited chromosomes which are responsible for the organization of the nucleolus. The continuity of the connexion of the nucleolus with the two chromosomes is evident in all stages of meiosis where the nucleolus is present. At diakinesis two bivalents are attached to the nucleolus (Pl. I, Figs. 2 and 4), or if there are two nucleoli, one bivalent is attached to each (Pl. I, Fig. 3). At interphase there are two nucleoli, to each of which one prochromosome is attached (Pl. I, Fig. 8), or these may fuse and two prochromosomes are then attached to the single nucleolus (Pl. I, Fig. 6). This condition is also borne out by the fact that in mitotic telophase four small nucleoli originate (Pl. I, Fig. 7) which later fuse into one.

There is also another question connected with the interpretation of the number of nucleoli that originate in the telophase, and therefore with the number of satellited chromosomes. De Mol (1926) examined seedlings of *Hya-cinthus*, and found that the number of nucleoli in the resting nucleus was correlated with the number of monoploid complements present. According to him the number of nucleoli in the cells was a reliable guide to the polyploidy of the plant, diploids having generally two nucleoli, triploids three, and tetraploids four. The simple nucleoli were all of the same size, the fusion nucleoli, when they occurred, being larger. Heitz (1931 *a*) also shared this opinion when he concluded that all plants must have satellited chromosomes, haploid with one, diploid with two, and so on. McClintock (1934) has also emphasized this correlation. The occurrence of two deeply staining bodies in connexion with two pairs of zygotene threads, the attachment of two bivalents to the

nucleolus in diakinesis, the presence of two nucleoli in interphase, the origin of four nucleoli in somatic telophase, and the attachment of four prochromosomes to the nucleolus of the archesporial cell of the ovule, all these point to the fact of the plant being a tetraploid though a functioning diploid. It has already been suggested in connexion with the secondary association that the plant is likely to be a secondarily balanced allotetraploid, and the nucleolar behaviour confirms this suggestion.

SUMMARY

The diploid chromosome number of *Gynandropsis pentaphylla* is thirty-four and the haploid seventeen.

The origin and behaviour of the megaspore is essentially the same as described for *Cleome Chelidonii*. The linear triad, which is the most commonly prevalent condition, is not held to be of any phylogenetic significance.

The origin and behaviour of the tapetum in the anther-sac is described. It is differentiated from the innermost wall layer and becomes binucleate early. The nuclei divide again and the resulting four nuclei fuse. Different cells of the same tapetal layer show different stages of division and fusion of their nuclei.

The pollen grains are binucleate at the time of shedding. The generative nucleus is surrounded by specialized cytoplasm with a thin membrane to form a lenticular cell.

Secondary association in stages of meiosis has been recorded and the basic number, based on maximum association, is found to be seven.

The behaviour of the nucleolus is described and two satellited chromosomes are found to be responsible for the organization of the nucleolus in the monoploid cell. From both secondary association and nucleolar behaviour, it is concluded that the plant is a secondarily balanced allotetraploid.

In conclusion, it is a source of sincere pleasure to record my indebtedness to Professor R. R. Gates for his helpful guidance and unfailing interest in the progress of the work.

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EXPLANATION OF PLATE I

T. S. Raghavan's paper on 'Morphological and Cytological Studies in the Capparidaceae. II. Floral Morphology and Cytology of *Gynandropsis pentaphylla* DC'.

Fig. 1. Pollen mother-cell with zygotene threads. Two pairs of chromosomes are attached to the nucleolus with the deeply staining bodies at the region of contact. $\times 3,400$.

Fig. 2. Early diakinesis. R = ring bivalents. Note the two bivalents attached to the nucleolus. $\times 3,400$.

Fig. 3. Early diakinesis. Note two nucleoli, to each of which one bivalent is attached. $\times 3,400$.

Fig. 4. Mid-diakinesis. The bivalents have shifted to the centre. Note the two bivalents attached to the nucleolus. $\times 3,400$.

Fig. 5. Prometaphase. Note their close aggregation as also the beginning of the secondary association. $\times 3,400$.

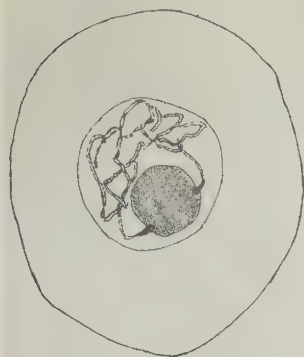
Fig. 6. Interkinesis. All the 17 prochromosomes are seen in the lower nucleus, two of which are attached to the nucleolus. $\times 3,400$.

Fig. 7. Somatic telophase. Note four nucleoli, to each of which one prochromosome is attached. $\times 3,400$.

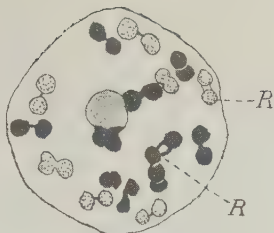
Fig. 8. Telophase preceding interkinesis. Note two nucleoli, to each of which one prochromosome is attached. $\times 3,400$.

Fig. 9. Tetrahedral arrangement of the microspore nuclei. $\times 3,400$.

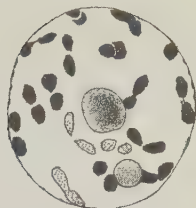
Fig. 10. Quadrate arrangement of the same. $\times 3,400$.



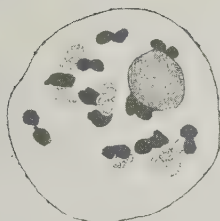
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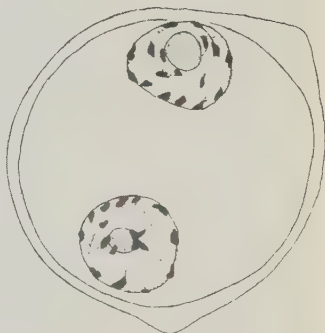
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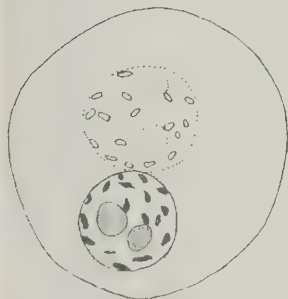
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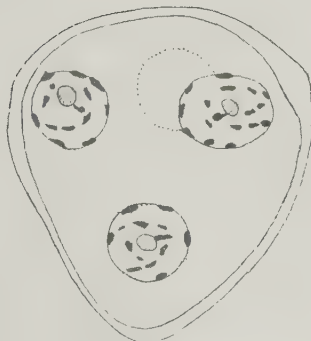
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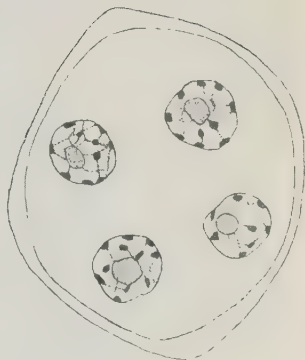
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The Occurrence of Long and Short Cycles in Growth Measurements of *Lemna minor*

BY

HUGH DICKSON

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With four Figures in the Text.

I. INTRODUCTION

DURING an investigation of the effects of alternating periods of light and darkness on the growth of *Lemna minor* (Dickson, 1938) it was found that the rate of frond multiplication was not strictly constant, i.e. that the logarithm of the frond number plotted against time did not give a straight line. It was thought at first that this divergence from the results of previous work on *Lemna* was due to the plants not having come into equilibrium with the conditions under which they were growing. Measurements were consequently continued for a further period, when it became apparent that the rate of increase in frond number, while approximately exponential, showed a variation which takes the form of a long-wave motion superimposed on the exponential curve. The time required for the completion of a cycle ranges from twenty-five to forty days according to the conditions. A short cycle has also been established, and the following account deals with various aspects of these two cycles.

2. EXPERIMENTAL RESULTS

The data given below are taken from certain of the experiments described in the paper referred to previously, Dickson (1938), in which cultural and other details are to be found.

(a) *The long cycle in frond-number determinations.*

The graphs of Figs. 1 and 2 show the differences between successive log. frond numbers and their respective calculated values plotted against time. In the four graphs A to D of Fig. 1, the culture solution was made up with distilled water condensed on copper. This was injurious and ultimately had a lethal effect; the equations of the rate of increase in number were calculated from measurements made during the first twenty-one days in each case, i.e. before injuries had become apparent. The four graphs E to H of Fig. 2 are from material grown in a solution made up with water condensed on glass, in which no lethal effect was produced, and in these cases the equations were

calculated from all the observations. The ordinates in all cases represent the differences between the log. frond numbers and their respective values calculated from the equations. The equations were all obtained by the method of

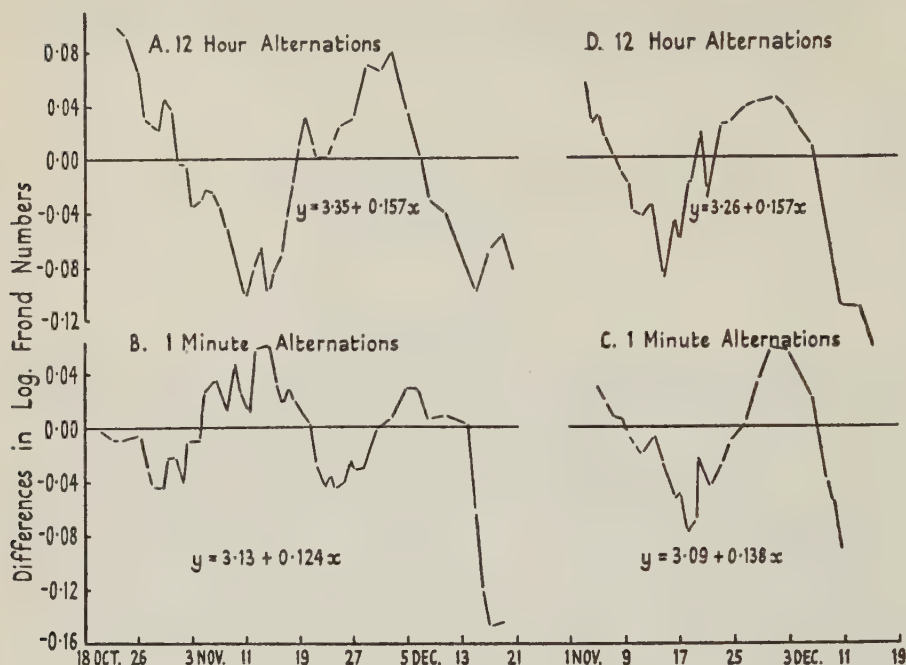


FIG. 1. For explanation see text.

least squares (Fisher, 1928), and are given below each graph. In all cases x represents the increase of the log. frond number in two days.

In Fig. 1 measurements were made every day for the first part of each experiment, but subsequently only on alternate days. In Fig. 2, on the other hand, measurements were carried out on alternate days, except over the period March 3–12 inclusive, when they were made every day. The four experiments in Fig. 1 were all carried out with *Lemna* obtained originally from Chelsea. A clone was developed and grown for a fortnight under the conditions of the experiment. Two samples of 100 fronds each were then taken from it and one placed under one-minute alternations of light and darkness (B), while the other (A) was kept in the twelve-hour alternations in which the plants had been growing. Measurements were then made on these colonies. After thirteen days samples were taken from each, that from the colony in the twelve-hour alternations being placed in the one-minute alternations and vice versa. Measurements were then made on these new colonies (respectively, C and D, in the figure) and were continued with the old ones. Of the four graphs in Fig. 2, E, F, and G were obtained from material similar to the earlier experiments, but in each case the colonies were started indepen-

dently of one another, material being obtained from the common stock (which was in a somewhat inactive condition) at arbitrary times and grown under standard conditions for fourteen days before measurements were begun.

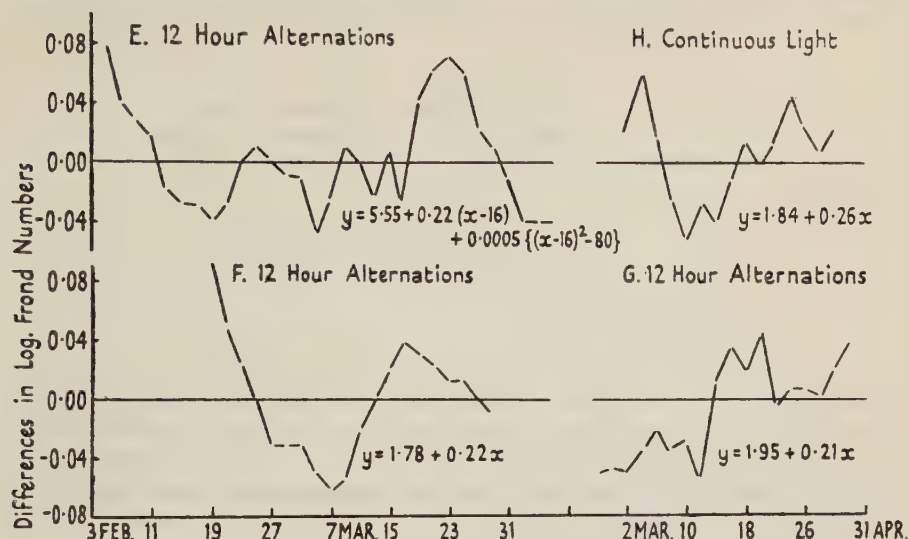


FIG. 2. For explanation see text.

The remaining graph (H) of Fig. 2 represents results with material obtained from Sutton and grown under continuous light.

All eight graphs of Figs. 1 and 2 have a common characteristic, namely, that the values rise and fall about the mean in a fairly regular manner. The curves E and G of Fig. 2 are the least regular, and it will be seen that this is due to the deviations of the experimental from the calculated values being too small between March 3 and 12. As pointed out above, measurements were made on successive days during this period after which observations on alternate days were resumed, and it seems possible that the irregularities are due to this cause. The values of the differences between the log. frond numbers found at certain wave crests and troughs and their respective mean calculated values expressed as percentages of the latter are shown in Table I. They are seen to range from a minimum of 14 to a maximum of 63 per cent.

Though there is little doubt from the results obtained that the variation does take a periodic form, it has not been possible owing to the time required to complete a wave-cycle to establish statistically a periodicity for the wave motion. The effect will in future be referred to as the long cycle to distinguish it from a short cycle to be referred to below. The length of the wave varies considerably between different experiments, the minimum being about twenty and the maximum forty days. There is some indication that the length of the wave is negatively correlated with the mean growth-rate, but the results are not sufficient to establish this.

It will be seen from the figure that the wave phase is altered on transferring plants from twelve-hour to one-minute alternations, and vice versa. Thus B, Fig. 1, is of material taken from A and put into one-minute alternating conditions and the phase has become the inverse of that shown by A, i.e. altered

TABLE I

Experiment.	Percentage increase of crest rate over mean rate.	Percentage decrease of trough rate below mean rate.
A	51	63
B	48	32
C	43	57
D	25	51
E	32	21
F	14	27
G	19	24
H	23	19

by half a wave-length. Again, material for C was taken subsequently from A and material for D from B. In both cases the phase has been advanced about a quarter of a wave-length following transfer to new conditions. Results to be published later indicate that the amount of alteration in the phase may depend on the particular phase of the material at the time of its transfer to new conditions.

(b) *The short cycle of frond-number determinations.*

Fig. 3 is based on measurements made on Lemna grown in a solution made up with distilled water condensed on copper, while in the experiments shown in Fig. 4 a glass still was used. Both figures are similar, in that they show the dry weight of Lemna, expressed in grammes per 100 fronds, plotted against time and the differences between successive log. frond numbers, summed in (overlapping) threes, against time. The object of each figure is to illustrate a short recurring cycle, though they also bring out the long cycle already referred to. The method of preparation of these graphs was as follows: Let a, b, c, d , and e be the log. frond numbers on successive days and consequently $b-a, c-b, d-c$, and $e-d$ the differences between successive pairs of observations, and let $2n$ days be the period of a complete cycle, then it can be shown mathematically that if the values $(b-a)+(c-b)+(d-c)+\dots$ to the n th term, $(c-b)+(d-c)+(e-d)\dots$ to the n th term, &c., satisfy a periodic function, the original values a, b, c , and d are also periodic. The effect of summing the differences covering periods of n days where n is half the period of a cycle is to accentuate the wave to the maximum extent. The values of n in Figs. 3 and 4 were obtained by trial and error, and the values 3 and 6 respectively were found to give the best wave curve.

In Fig. 3 observations were made on successive days, in Fig. 4 on alternate days, so that summing differences in *threes* in each case involved periods for each point on the curves of three and six days respectively. This in Fig. 3

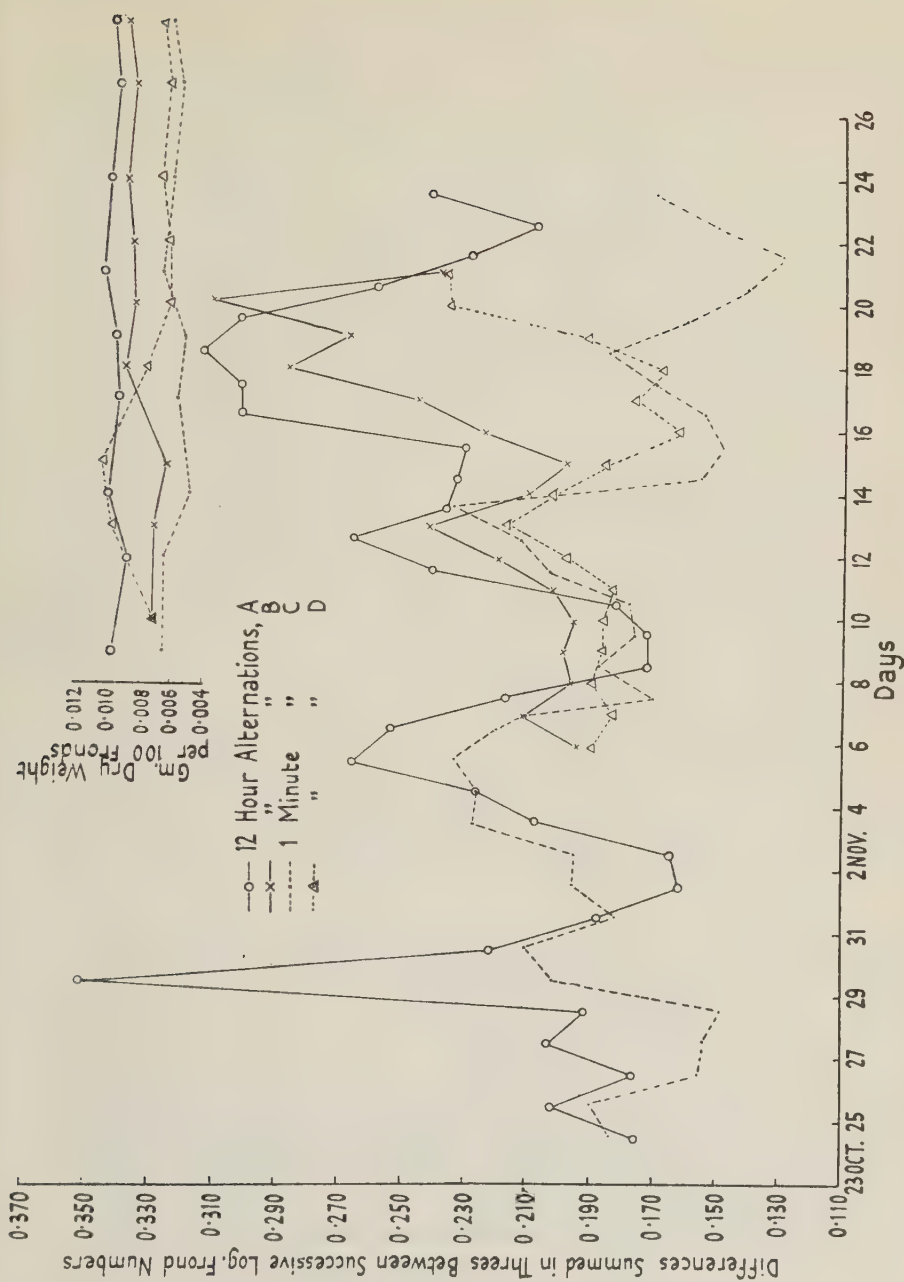


FIG. 3. For explanation see text.

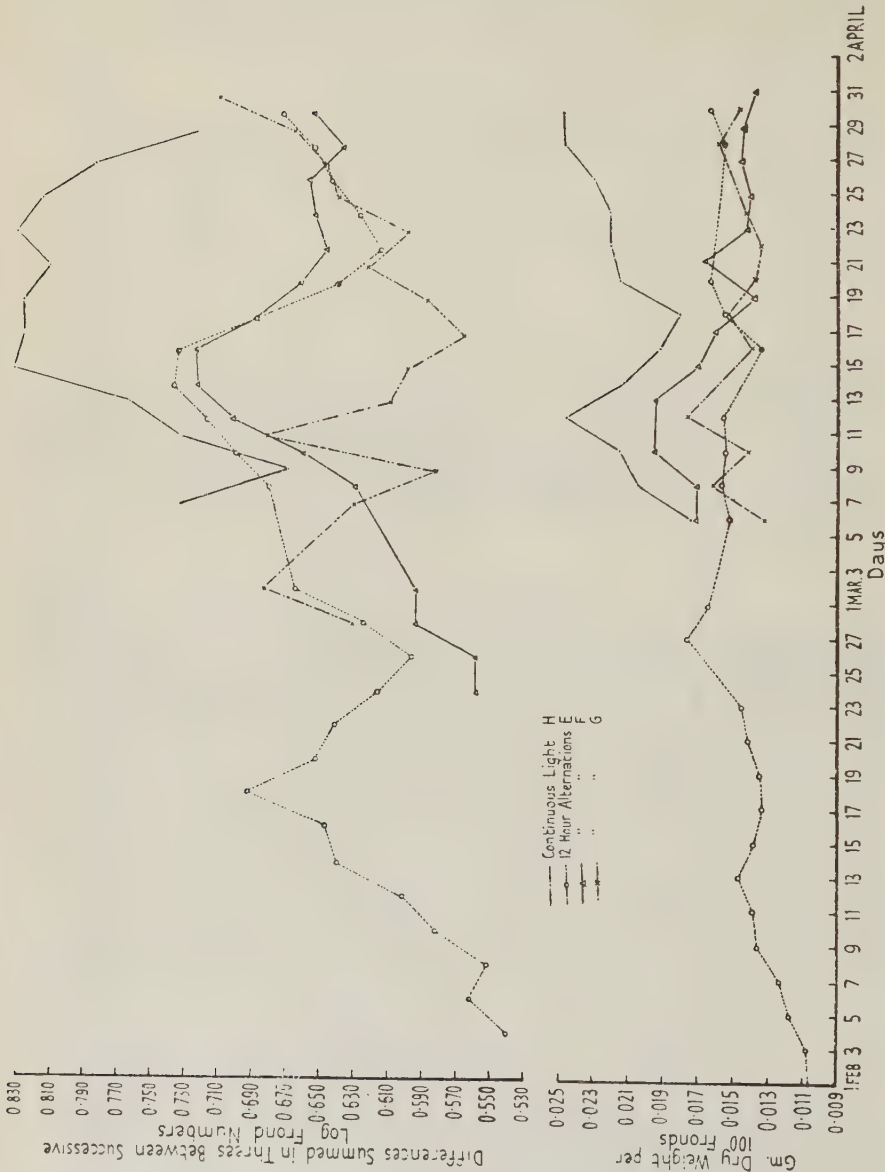


FIG. 4. For explanation see text.

indicates a cycle of six days' duration which fits the curves satisfactorily. In Fig. 4, however, each wave obviously has a *four-day* period, see especially the curve E for twelve-hour alternations Feb. 3 to March 2. If the log. frond numbers on alternate days were a, b, c, d , &c., then the values actually plotted in Fig. 4 would be $(b-a)+(c-b)+(d-c)$, $(c-b)+(d-c)+(e-d)$, &c., that is $(d-a)$, $(e-b)$, $(f-c)$, &c. If a represents a value at the trough of a four-day wave, then d will be that at the next crest but one. As the different log. frond numbers are not independent of one another, the result of summing the differences in threes (i.e. in six-day periods) has been to smooth out errors by bringing four observations into the calculation of each difference in place of the two which otherwise should have been used in a four-day cycle. In Fig. 3 the waves of each of the four sets of results are regular in that their respective peaks and troughs occur in all cases at approximately the same time. This indicates that while transference from one set of light conditions to another may affect the phase of the long wave-cycle as already shown, it does not necessarily do so where the short cycle is concerned.

A consideration of the curve E of Fig. 4 will show that, having allowed for the dominant influence of the long wave on the short one where the former's crests and troughs occur, each point on the curve is alternately above or below a line joining the points on either side of it. The only exception among the fourteen observations up to March 2 is that on February 26, where the point should be above such a line, but is below, and this point is at the trough of the big wave. The same is true from March 8 to the end of the experiment, the exceptions being on March 16 and 22, where again the long wave is respectively at the crest and trough of its phase. Since two observations are omitted, between March 2 and 8, i.e. those on the 4th and 6th, it will be seen that the cycle is continuous throughout the experiment, i.e. for sixty days. Apart, then, from the three values influenced by the phase of the long wave and the two blanks in the middle of the experiment, the odds against such an alternation of high and low values being due to chance are in the ratio of 1 in 2^{30} .

A consideration of the remaining three curves of Fig. 4 indicates that while they do not show such a regular periodic effect as that of E, there are few exceptional points. Thus in curve H formed of twelve points, only one, namely, that on the 25th, is not in conformity with the four-day cycle. This point is too high and is again near the crest of the long wave. In the case of F there are two points out of the cycle—those on the 20th and 28th. The former is below (instead of above) the mean of the two adjacent points by less than 1 per cent. of its value, and the latter is also too small.

Taking 0.157 (A and D) as a measure of the rate of increase of log. frond number in two days when the short cycle is of six days' duration, and 0.218 (E and F) as the rate with a four-day cycle, it will be seen that the relation between the relative rates of frond number increase in the two treatments ($218/157$ or 1.4) is approximately inversely proportional to the ratio of the

respective lengths of their cycles ($4/6 = 0.66$). It is suggested that results at other growth-rates might show that the length of the short cycle was a simple function of the rate of growth, within certain limits.

(c) *The relationship of the dry-weight to the long and short cycles displayed by the log. increase in frond number.*

The dry-weight determinations do not throw much light on the short cycle. Each point is based on only one measurement of weight, whereas as already shown, several counts are included in computing each point of the frond number curves. In the latter case the first eleven points of E, Fig. 4, show an average deviation from the curve of closest fit, drawn freehand through them, of about 1.1 per cent. of their mean value. It is therefore not to be expected that the dry weights would serve to illustrate such small deviations. It may, however, be pointed out that in the dry-weight curve for E, the first eleven points with the exception of that on the 17th, are in exact agreement with expectation based on a four-day cycle.

The dry-weight measurements have, on the other hand, a very definite bearing on the long cycle. In almost all cases where there is a crest in the long wave of a frond-number curve there is a trough in the corresponding dry-weight curve. In other words, the dry weights also show a long cyclic variation which is in inverse phase to that of the frond numbers. The curve E brings this out most clearly. In other curves of Fig. 4 the crests and troughs coincide approximately and are seldom more than a day out. In Fig. 3 the chief discrepancies are to be found in C and D, in both of which curves the material had been transferred to new conditions and is obviously (*vide* the sudden marked increase in weight of D eight days after being transferred to one-minute alternations) in course of becoming adapted to them.

3. DISCUSSION

While the rate of increase in frond number is approximately exponential over a long period of time, it has been shown that it is subject to minor fluctuations which have in previous work been attributed to experimental error. Dry-weight determinations have similarly been found to fluctuate periodically. The chief deviation from an exponential increase in frond number and in the regularity of dry-weight determinations is found in a long variation which takes the form of a 'wave' in both instances. It has been impossible to establish definitely the periodicity of the wave-cycle owing to its length, twenty-five to forty days, but from parallel experiments there seems to be little doubt that such a periodic cycle is present. Superimposed again on the long variation is a short cycle which has been definitely established. The wave-lengths in both sets of determinations (i.e. frond-counts and dry-weight determinations) are of approximately equal length in any one experiment but are in opposite phase.

Table II gives the rates of increase in log. frond number reckoned over

periods of seven days, and the differences between these and the mean rate as determined for the whole experiment and expressed as percentages of the latter. The rates represent the increase in log. frond number in two days. The seven-day periods were selected so as to embrace the respective up and down slopes of the long wave, that is, the times at which the rates of frond increase were at their maxima and minima respectively. The + signs apply to the up, and the — to the down slopes of the wave.

TABLE II

Experiment.	Rates during 7 days.	Variation as per cent. of mean rate.	Experiment.	Rates during 7 days.	Variation as per cent. of mean rate.
E	+0.23	5.5	B	+0.14	12.9
	—0.19	12.8		—0.10	19.5
F	+0.23	5.5	A	+0.19	21.0
	—0.19	12.8		—0.14	8.3
H	+0.29	10.7	D	+0.17	8.3
	—0.23	12.0			

It will be seen from the table that it is possible to introduce an error amounting to ± 20 per cent. of the mean rate of increase of log. frond number, by neglecting to take into consideration the phase of the long wave.

Assuming a cycle of about twenty-eight days for the long variation, rates determined from measurements extending over more than seven days will be liable to smaller errors than the above. Where the period is under seven days the error should not be much larger, but the chances of introducing the maximum error will be greater. The error due to the short cycle will be negligible even with measurements extending over only three or four days.

A second form of error due to the long wave will be introduced where the wave-phase of the experimental plants is altered as a result of introducing the plants to the conditions under which the growth-rate is to be determined and where the rates of the control and experimental sets, measured simultaneously, are then compared. This error may in certain circumstances be as much as 40 per cent. of the mean relative rate of frond increase. It has been found that different periods of alternating light and darkness alter the wave-phase, the degree of change in phase probably being influenced by the particular phase of the long wave at the time of removal to the new environment. (See Fig. 2.) Observations on dry-weight determinations show that they are subject to errors due to the long wave in much the same way as are the determinations of relative rate of frond increase.

As regards factors concerned with the long and short cycles little is known. It has been shown, however, that the lengths of both cycles are probably functions of the mean relative rate of frond increase. It is thought probable that the rhythmic increase in frond number and variation in dry weight may be related to the fact that the growth data are those of colonies of numerous individuals, i.e. that while these particular cycles are exhibited by the colonies

they are not shown by the plants taken singly. This aspect of the matter is now undergoing investigation.

4. SUMMARY

The data used are taken from certain of the experiments on *Lemna* described in a previous paper in which rate of frond increase and the dry weights were determined under different lighting conditions for continuous periods of up to nine weeks.

A long variation in the relative rate of frond increase, taking the form of a wave, and a short cycle are described. The length of the latter cycle, and probably also that of the former, are functions of the mean rate of frond division. Removal of plants from one light environment to another was found under certain conditions to alter the phase of the long cycle, the phase under the new environment being the inverse of that in the old. Dry-weight determinations gave results complementary to those obtained with frond counts.

It has been shown that the long cycle may introduce an error of as much as 20 per cent. in rate of increase of log. frond number, and an error of 40 per cent. is possible should the control and experimental plants be in opposite phase when measured.

It is suggested that experiments based on the fact that the culture of *Lemna* used in experimental work is a colony of plants and not an individual, may help to solve the problem as to the factors conditioning both the short and long cycles.

The author has pleasure in thanking Professor V. H. Blackman for the encouragement and advice he has so generously offered during the period of these experiments.

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Cytogenetical Studies in the Oryzeae

I.¹ Chromosome Studies in the Oryzeae

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With Plate II and thirty-one Figures in the Text

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INTRODUCTION

THE subdivision of the grasses into sub-families, tribes, and sub-tribes has presented considerable difficulties to systematists, who differ a great deal in the details of their arrangement in any phylogenetic series. Brown (quoted by Avdulov, 1931) was the first to recognize two main sub-families, the Panicoideae and the Pooideae, and this system is even now followed, with slight modifications introduced by later workers.

The earliest classifications were based on external morphology alone. Later, however, the anatomical characters of the leaf and the nature of starch grains—whether compound or simple—came to acquire a taxonomic value. The karyological method of classification of the Gramineae was first undertaken by Avdulov (1931). He determined the somatic chromosome number for a large number of genera and proposed a system of classification based on

¹ Part of thesis approved for the Degree of Doctor of Philosophy in the University of London.

a monophyletic origin of the family. Later Hunter (1934) added a few more chromosome numbers to the necessarily incomplete list compiled by Avdulov and concluded that his results in the main confirmed Avdulov's conclusions.

The Oryzeae are an isolated group in the Gramineae without any very obviously close connexions. In some respects they are primitive, as for instance in some of their members having six stamens; and in others they are advanced, as in the spikelets being reduced to the one-flowered condition and the glumes entirely suppressed or replaced by two minute empty lemmas. Not only are they somewhat isolated floristically, but they are also very different ecologically, for they have responded to a very different environment from the majority of grasses. Except *Lygeum spartum*, which grows on rocky soil, all the other genera have become adapted to a hygrophilous or purely aquatic mode of life. Because of this peculiar mixture of advanced and primitive characters in the tribe, they have been differently classified by systematists. Bentham and Hooker, and Hackel (quoted by Avdulov, 1931) included them among the Panicoideae because of the suppression of the glumes, and Stapf and Hitchcock (quoted by Avdulov, 1931) included them in the Pooideae because of their laterally compressed spikelets. Prat (1932), on examination of the epidermal hairs of *Oryza* and *Lygeum*, thought they belonged to the Panicoideae because they had bicellular hairs in common with the latter. While the position of the tribe in the family is still doubtful, the genera composing them are also uncertain. Various genera like *Pharus*, *Leptaspus*, *Streptochaeta*, *Anomochloa*, and *Lygeum*, which were at some time or other included in the Oryzeae, have now been removed from them on morphological grounds (Hutchinson, 1934). Hutchinson in his recent book has classified the Oryzeae into two sections; (1) the Oryzinae, including three genera, viz. *Oryza*, *Leersia*, and *Hygroryza*, and (2) Zinaniinae, including four genera, viz. *Zizania*, *Zizaniopsis*, *Hydrochloa*, and *Luziola*. The Oryzinae have hermaphrodite spikelets which are laterally compressed and keeled, while the Zinaniinae have unisexual spikelets which are more or less terete or slightly compressed.

From a karyological standpoint the Oryzeae have not received much attention. Cytological studies in the past have been practically confined to only one species of the genus *Oryza*, viz. *O. sativa*, the cultivated rice. Kuwada (1910) was the first to determine its chromosome number as $2n = 24$. Later Rao (1929), Selim (1930), Kato (1930), and Nandi (1936 a, b) examined several varieties of the same species and confirmed Kuwada's results. Nandi (1936 b) has also recorded the chromosome number of *O. officinalis* and *O. minuta* to be $2n = 24$ and $2n = 48$ respectively. Avdulov (1931) also examined only *O. sativa* and found that the somatic complement was composed of twenty-four chromosomes, of which one pair had well-marked knobs. The presence of only two such chromosomes in the somatic complement led him to suppose that the basic number for the species was twelve. Although he realized that evidence from one genus was inadequate, he thought

that other genera in the tribe would be characterized by a similar karyotype on account of certain similarities among them in anatomical characters. On the presumption, therefore, that the basic number for the tribe is twelve, he included *Oryza* in a special section of the sub-family Poateae (corresponding to Pooideae), which included a heterogeneous collection of genera bound together by superficial resemblances but had twelve small chromosomes as the basic number.

Recent work, however, of Nandi (1936 *b*) and Sakai (1935) does not support the conclusion of Avdulov with regard to the basic number of *O. sativa*. These two authors, from a study of secondary association of chromosomes at meiosis, have come to the conclusion that the basic number for the species is five. The presence of only two satellited chromosomes in the somatic complement of the variety examined by Avdulov is not incompatible with the fact that rice is a secondary tetraploid with a basic number five. It has been shown in another paper (Ramanujam, unpublished, *a*) that the number of satellited chromosomes may be modified in polyploid species by a variety of circumstances and as a result of such modifications in *Oryza sativa*, varieties with two and four satellited chromosomes may be found. Nandi (1936 *b*) has actually seen four satellited chromosomes in the somatic complement of certain varieties. I have actually noticed varieties in *Oryza sativa* with two nucleoli and four nucleoli at somatic telophase, which may represent varieties with two and four satellited chromosomes respectively.

With a view to obtaining more evidence regarding the basic number of chromosomes for the tribe, their correct classification and position in the family, a chromosome survey of the whole tribe was undertaken. A preliminary report of the results was published (Ramanujam, 1936 *c*). The investigation reported in this paper is necessarily incomplete. It nevertheless points to some definite conclusions regarding the basic number and phylogeny of the tribe. The work will be further continued as more material comes to hand and will be followed up by cytogenetical studies of interspecific and intergeneric hybrids, which should lead to a closer understanding of relationships and origin of species in the tribe to which the cultivated rice, viz. *Oryza sativa* belongs.

ORIGIN OF THE MATERIAL

Seeds of several varieties and species of the different genera were obtained from several places by Professor R. R. Gates, to whom my thanks are due. My thanks are also due to Mr. K. Ramiah, Paddy Specialist, Coimbatore, for seeds of several varieties of *Oryza* species; Mr. H. C. Sampson, Economic Botanist, Royal Botanic Gardens, Kew, for seeds of a large number of varieties of *Oryza sativa* and *O. glaberrima*, collected in Africa; Dr. T. K. Koshy, Professor of Botany, Science College, Trivandrum, for fixed material of root tips of *Hygroryza aristata*, and Dr. Weatherwax of Indiana University, for material of *O. latifolia*, *Zizaniopsis miliacea*, *Leersia hexandra*, and *L. oryzoides*.

Two varieties of *Zizania aquatica* were kindly sent by the Bureau of Plant Industry, Washington, who made arrangements to keep the seeds moist at a low temperature in the ship's refrigerator. They germinated *en route* and have since been grown successfully. I am also thankful to Lady Davy, who supplied live plants of *Leersia oryzoides* from the Basingstoke Canal. The following is a full list of material obtained for study, arranged according to the classification of Hutchinson (1934):

Zizaniinae.

Zizania aquatica L. (three varieties).

Zizania latifolia Turch. (cutting obtained from Kew Gardens).

Zizaniopsis miliacea Doell and Aschers.

Oryzinae.

Oryza sativa L. (several varieties).

Oryza glaberrima Steud. (several varieties).

Oryza officinalis Wall. (three varieties).

Oryza Barthii Cheval. (one variety).

Oryza longistaminata Cheval and Roeh. (one variety).

Oryza latifolia Desv. (one variety).

Oryza minuta Presl. (two varieties).

Leersia hexandra Sw. (two varieties).

Leersia oryzoides Sw. (two varieties).

Hygroryza aristata Nees. (one variety).

Lygeae (not included in the Oryzeae).

Lygeum spartum L.

Except *Zizaniopsis miliacea* and *Oryza latifolia*, whose seeds did not germinate, and *Hygroryza aristata* in which case only fixed material was obtained, plants of other species were grown at the Courtauld Genetical Laboratory, Regent's Park, London, during the summer of 1936. Root tips were fixed in all cases for determination of chromosome numbers. Confirmation of counts of somatic chromosomes was obtained from meiosis wherever material for study was available.

CYTOLOGICAL TECHNIQUE

Root tips were fixed in 2 BE or 2 BD for twenty-four hours, washed, dehydrated, and embedded by the chloroform method in 52° wax according to La Cour's schedule (La Cour, 1931). Sections were cut at 12–18 μ thick, bleached in a mixture of three parts of 95 per cent. alcohol and one part of 20-volume hydrogen peroxide for twenty-four hours and stained by Newton's iodine gentian violet technique.

Pollen mother-cells were fixed in Navashin's fluid or La Cour 2 BE for twenty-four hours after immersion in Carnoy for a few seconds. The fixed material was washed, dehydrated, embedded, and sectioned in the same way

as root tips, except that slides were mordanted in 1 per cent. chromic acid solution for about fifteen minutes before staining.

Aceto-carmine smears were employed to determine right stages of fixation.

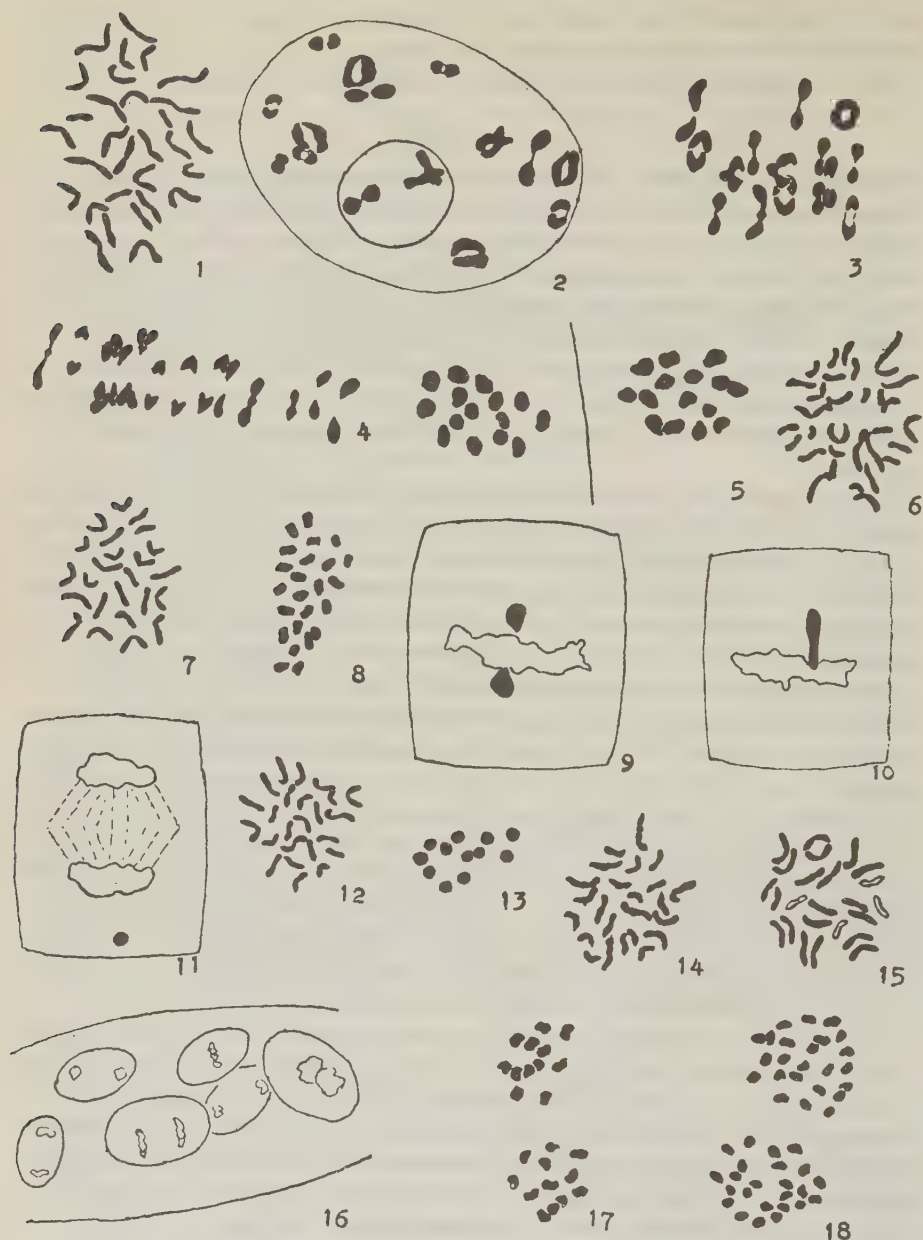
OBSERVATIONS

Zizaniinae. The genus Zizania. Two species of this genus were examined for chromosome number for the first time. *Zizania aquatica*, known as Indian wild rice, is common over extensive areas in northern and eastern states of America. Seeds of two varieties of this species obtained from the Bureau of Plant Industry, Washington, showed thirty chromosomes each in root-tip mitosis. A third variety, originally obtained from America, growing at the Kew Gardens, was also examined and found to contain thirty chromosomes at somatic metaphase in root tips. The somatic complement of one of the varieties is illustrated in Text-fig. 1. The chromosomes are bigger than those of *Oryza*.

The plants of the two varieties growing at Regent's Park have not yet flowered at the time of writing this paper. A few preparations of dividing pollen mother-cells were, however, made from the scanty material available at Kew. At diakinesis the chromosomes were found as fifteen bivalents arranged peripherally in the nuclear area, two of which appeared to be associated with the nucleolus. In some cases the bivalents were so loosely paired that the two chromosomes seemed quite separate (Text-fig. 2). In several cells examined at this stage, no associations of more than two chromosomes were noticed. At metaphase the fifteen bivalents arrange themselves regularly on the equator and the anaphasic separation proceeds regularly, with some of the loosely paired chromosomes disjoining earlier than the other pairs. Text-figs. 3 and 4 illustrate side-views of metaphase and early anaphase respectively. At metaphase II (Text-fig. 5) fifteen chromosomes are seen regularly in each daughter-cell. After a normal division II, four equal spores are formed in each mother-cell. No abnormality of division was observed in the material examined. Observations of secondary pairing in the material could not be made owing to insufficient material.

Z. latifolia is an Asiatic species, known commonly as Manchurian wild rice. This species, originally obtained from Siberia, is growing at Kew Gardens and is not known to have flowered in the open since it was obtained two years ago. Rhizomes of this plant were grown in the tropical house at Regent's Park last summer without success in inducing them to flower. Chromosome number of the species was obtained from somatic mitosis in root tips. It had $2n = 30$ chromosomes (Text-fig. 6), the same as that for *Z. aquatica*. The presence of thirty chromosomes in *Zizania* affords additional evidence to the fact that the basic number for the tribe is five and not twelve.

The genus Zizaniopsis. Z. miliacea. Seeds of this species obtained from Indiana, U.S.A., through Dr. P. Weatherwax, failed to germinate and hence its chromosome number could not be obtained.



TEXT-FIGS. 1-18. All drawings were made at bench level with the aid of a camera lucida. An apochromatic objective N.a. 1.3 was used in conjunction with Zeiss eyepiece K 30 giving an approximate magnification of 4,200 diameters. In the case of Text-fig. 16, eyepiece K 6 was used giving an approximate magnification of 900 diameters. Fig. 1. *Zizania aquatica*. Somatic metaphase ($2n = 30$). Fig. 2. *Zizania aquatica*. Diakinesis. Fig. 3. *Zizania aquatica*. Side view of metaphase I. Fig. 4. *Zizania aquatica*. Early anaphase I. Fig. 5. *Zizania*

Arrangements are being made to obtain material of *Hydrochloa* and *Luziola*, the other genera belonging to *Zizaniinae*. Chromosome numbers of these genera, when determined, should give definite information regarding the basic number of this section. It is likely that this section, which forms a natural group according to the systematists, may be characterized by the basic number five.

Oryzinae.

The genus Oryza. The species *Oryza sativa*, the cultivated rice, has been studied cytologically by several workers (Kuwada, 1910; Rao, 1929; Kato, 1930; Sakai, 1935; and Nandi, 1936). All the varieties examined so far have $2n = 24$ chromosomes. The chromosomes are fairly small, ranging in size from $0.7\ \mu$ to $2.8\ \mu$. Avdulov (1931) records that he observed two chromosomes in the somatic complement with well-marked knobs, which are probably the satellited chromosomes. Nandi (1936 *b*) has actually observed four satellited chromosomes in the somatic complement of the variety he examined. Secondary association of chromosomes at meiosis has been studied by Nandi (1936 *b*) and Sakai (1935). A maximum association of five groups consisting of two groups of three bivalents and three groups of two bivalents was observed by both the writers, who concluded on this evidence supported by other evidences of the presence of polymeric factors (Matsuura, 1933) and autosyndesis (Sakai, 1935, and Ichijima, 1934) that the original basic number of the species is five and that the number twelve has been derived as a result of secondary allotetraploidy.

I examined the somatic chromosomes of five varieties of *Oryza sativa* obtained from different localities, viz. Africa, India, China, and Japan, and all of them had the same number, $2n = 24$. The chromosomes are small and more or less similar in all the varieties. A morphological study of the somatic complement was very difficult owing to the smallness of the chromosomes, and even the presence of satellites could not be made out in many excellent preparations. Text-fig. 7 and photomicrograph Plate II A illustrate the somatic chromosomes of one of the varieties examined.

aquatica. Metaphase II. Fig. 6. *Zizania latifolia*. Somatic metaphase ($2n = 30$). Fig. 7. *Oryza sativa* (variety T 24). Somatic metaphase with normal chromosomes (same as photomicrograph Plate II A). Fig. 8. *Oryza sativa* (variety T 24). Somatic metaphase with highly condensed chromosomes (same as photomicrograph Plate II B). Fig. 9. *Oryza sativa*. Mitotic metaphase with the nucleolus divided into two halves on the plate. Fig. 10. *Oryza sativa*. Mitotic metaphase with the nucleolus constricted to divide unequally. Fig. 11. *Oryza sativa*. Mitotic telophase with nucleolar fragment lying outside the reforming nucleus. Fig. 12. *Oryza glaberrima*. Somatic metaphase ($2n = 24$). Fig. 13. *Oryza glaberrima*. Polar view of metaphase I with twelve bivalents. Fig. 14. *Oryza officinalis*. Somatic metaphase ($2n = 24$). Fig. 15. *Oryza officinalis*. Somatic metaphase showing somatic pairing of chromosomes (same as photomicrograph Plate II C). Fig. 16. *Oryza officinalis*. A portion of another locule showing two big tetraploid cells and four small diploid ones. Fig. 17. *Oryza officinalis*. Anaphase I in diploid pollen mother-cells with twelve chromosomes towards each pole. Fig. 18. *Oryza officinalis*. Anaphase I in tetraploid pollen mother-cells with twenty-four chromosomes towards each pole.

The meiosis in the different varieties proceeds regularly with the production of normal tetrads. A detailed description of the process is given by Nandi (1936 *a*). Secondary pairing was observed in the material studied and it ranged from no association to the maximum association in different cells.

Several hundreds of varieties of *O. sativa* are known and crosses between them succeed fairly easily, with normal Mendelian segregation of characters in the progeny (Matsuura, 1933). It is possible that these varieties arose as a result of genic differentiation of their chromosomes.

(*a*) *Mutation for chromosome size in O. sativa*. Two cells in a certain section of the root tip of the variety T 24 from Coimbatore showed an interesting abnormality of the chromosomes at mitotic metaphase. The chromosomes in these abnormal cells were very much condensed and appeared more like meiotic chromosomes. Text-fig. 8 and photomicrograph Plate II B illustrate metaphase in one of these cells, and Text-fig. 7 and photomicrograph Plate II A illustrate metaphase in a normal cell lying close to the former in the same section.

Different sources of variations in size and shape of chromosomes are identified in fixed preparations. These may be caused by external influences or by genetic causes. The variation in size of chromosomes recorded by Navashin (1934) in embryonic and adult roots and in different regions of the same root perhaps belong to the first category. Other cases, such as the characteristic variation between species and between varieties of the same species, are assumed to be due to genotypically controlled differences between the varieties. For example, two clones of *Fritillaria ruthenica* from different sources reported by Darlington (1936) were found to differ in the size of the chromosomes, the smaller type being smaller than any others in *Fritillaria*. Other cases of such genotypically controlled variations in size of chromosomes have been fully dealt with in another paper (Ramanujam, unpublished, *b*). Cases of such marked variation in size as is found in the present case within a single preparation from one plant are rather rare.

Such variations may be due to the differential effect of environment in different cells or may be due to genotypic difference between cells resulting from genemutation. Meurman (1928), in sterile *Ribes* hybrids, found that in certain pollen mother-cells in which the chromosomes remained unpaired, the chromosomes were bigger than those in others. These cells were situated peripherally in the anther locule and were larger, and Meurman thinks that such a condition may be due to special nutritional conditions in those cells. Darlington (1936), in his studies of *Fritillaria*, found two isolated abnormal pollen mother-cells at meiosis. In one cell of *F. pluriflora* the chromosomes were more condensed than usual and widely separated on the plate, more so than in any other pollen mother-cell. In the second case a pollen mother-cell in *F. meleagris* had chromosomes nearly as long as at mitosis and the nucleolar constrictions were still visible in them. He thinks that genotypic differences in these cells may have been responsible for the abnormal chromosomes. It

is possible that the extremely condensed nature of the chromosomes in the present case may have resulted from mutation in these cells, as adjacent cells showed normal chromosomes and therefore no difference in environment could be assumed. This is significant in connexion with the fact that different sizes of chromosomes in related species may be due to genotypically controlled differences in the different species. It is possible that size-differences of somatic complements in the different genera of the Oryzeae are merely due to such genotypic causes. This point will be referred to again later in the paper.

(b) *The behaviour of the nucleolus in mitosis in the variety T 24.* The behaviour of nucleolus in mitosis and meiosis has been the subject of several investigations in the past. While the problem of its origin is fairly well understood its composition and function is still largely a matter of conjecture. Gates (1937) has recently reviewed the literature about the origin of the nucleolus. The role which has been assigned to it in the economy of the cell has been quite varied and the literature dealing with it is quite extensive. Ludford (1922), Wilson (1928), Sharp (1934), Zirkle (1928), and Frew and Bowen (1929) have summarized these divergent views.

The nucleolus, which is usually formed in the telophase nucleus at mitosis, usually disappears with the dissolution of the nuclear wall and the onset of the metaphase at the subsequent division. However, in certain cases, the nucleolus has been observed to persist till late in mitosis and undergo a division followed by the polar migration of the divided halves. Examples of these cases both in lower and higher plants are given by Frew and Bowen (1929). These authors observed in certain cells of *Cucurbita pepo* at mitosis, that the nucleolus came to lie on the metaphase plate, assumed a dumb-bell shape, and separated into two fragments which migrated to opposite poles of the spindle. They also found that the nucleolar fragments moved to the poles prior to the chromosomes and were not included in the daughter nuclei at telophasic reconstruction but degenerated in the cytoplasm. The authors have discussed this behaviour of the nucleolus in terms of the mechanism of anaphasic movements. They are of opinion that the spindle area represents a region in which are localized the forces which are responsible for anaphasic movements and that the latter operate regardless of the nature of the bodies which find themselves in the spindle region—whether chromosomes or nucleoli. Zirkle (1928), who worked with specific nucleolar fixatives, adduces evidence that in *Zea mays*, part of the nucleolar substance, which is plastin, passes into the chromosomes at mitosis and the other part divides at metaphase and the divided fragments pass to opposite poles. The divided halves reached the poles earlier than the chromosomes and fragmented with practically all the pieces passing out into the cytoplasm. He sees in this behaviour of the nucleolus at mitosis a mechanism by which hereditary stimuli are transmitted from the chromosomes to the organism. He also observes that plastin, being electro-positive, changes the electro-negative spireme by flowing into it, to an electro-positive chromatin complex; thus the chromatin which

had collected at the equatorial plate as far as possible from the poles of the spindle, reverses its motion with its electrical charge and migrates to the poles. The persisting nucleolar material is also attracted to the poles because they are electro-positive.

In T 24, in some of the cells at mitosis the nucleolus was found to behave in the same way as in *Cucurbita pepo*. The nucleolus was found to lie between the chromosomes in the centre of the spindle, becoming elongated with its long axis parallel to that of the spindle. Soon it assumed a dumb-bell shape and separated into two halves which moved to opposite poles. The orientation of the nucleolus on the metaphase plate was found to vary in different cells. As a result, in some cells it divided into two equal or in some others into two unequal halves. Occasionally it was left outside the plate, in which case it did not divide but moved as such to one pole. Text-fig. 9 shows the nucleolus divided into more or less equal halves on either side of the equatorial plate, and Text-fig. 10 illustrates a cell in which it may divide into two unequal halves. The movement of the nucleolus to the poles, in every case, was found to be in advance of the chromosomes. Its size appeared to diminish during its movement to the poles where it was left out of the daughter nuclei, ultimately to degenerate. Text-fig. 11 shows the nucleolus lying away from the reforming telophasic nucleus. The division of the nucleolus on the equator and its movement to the poles do suggest the presence of forces at the spindle region which are responsible for such movements. A fuller understanding of the nature and significance of these forces in the anaphasic movements must await a more detailed study than has been attempted here.

O. glaberrima. This species is very similar in morphological characters to *O. sativa* and is largely cultivated in Africa. The somatic chromosomes of three different varieties were examined in root tips and were found to be more or less similar in size to those of *O. sativa*. The three varieties all had $2n = 24$ chromosomes. The somatic complement of one variety is illustrated in Text-fig. 12.

At meiosis, the chromosomes in pollen mother-cells form twelve bivalents and separate regularly at division I. Division II is also equally regular and four equal spores are formed at the end of the two divisions. Secondary pairing of chromosomes was noticed in this species as well. Polar view of metaphase I is shown in Text-fig. 13.

Crosses between this species and *O. sativa* succeed very easily, and many of the characters are segregated in Mendelian fashion in the progeny. The glabrous nature of the glume, on which the specific difference between the two species is based, has been found to follow a multiple factor segregation in crosses studied at the Paddy Breeding Station, Coimbatore (unpublished records of the station). It is probable that this species is only a variety of *O. sativa*.

O. officinalis. It is a perennial found in the wild in parts of Burma, Malaya, &c. It is often confused with *O. minuta*, which it closely resembles

in habit and nature of glumes. The chromosome number for this species has been recorded by Ramiah (1936) and Nandi (1936 *b*) as $2n = 24$. The somatic chromosomes of three different varieties of this species obtained from different sources were examined and found to be $2n = 24$ in each case. The chromosomes were slightly larger as a whole in the somatic complement than in *O. sativa* (Ramanujam, unpublished, *b*). Text-fig. 14 illustrates a normal somatic metaphase in one variety of this species. The metaphase pattern in many cells was characteristic, the chromosomes being sorted out in pairs and lying near each other without being in actual contact. The degree of this somatic pairing between homologous chromosomes was variable in different cells, ranging from a few pairs to nearly all pairs. Text-fig. 15 and photomicrograph Plate II c show a metaphase in which nearly all chromosomes are paired. Evidence of somatic pairing was found by Kuwada (1910) in *O. sativa*, although Nandi (1936 *a*) states that it was not found in his material.

At mitosis in the diploid in many Diptera the chromosomes are seen to lie close together in pairs (Metz, 1916; Koller, 1934). A paired condition of the chromosomes was first found in the somatic tissues of plants by Strasburger in 1905 (quoted by Gates, 1912). This condition has also been reported in several other plants; as in *Oenothera* (Gates, 1912), *Matthiola* (Huskins 1932), *Dahlia* (Lawrence, 1931), &c. The very characteristic arrangement of the chromosomes in pairs in all these cases cannot be an accidental one but must be due to some specific attraction due to homology between the paired chromosomes, which according to Gates (1911) may exist between parental chromosomes throughout the life-cycle of the sporophyte. Koller (1934) assumes that this somatic pairing may be conditioned by the genotype controlling the degree of attraction between homologous chromosomes. He observed that during somatic pairing each chromosome is composed of two sister chromatids. On the principle that pairs of paired chromatids repel each other, the two homologues which lie side by side ought to repel each other because they are double, each of them being composed of two chromatids. In the cases where somatic pairing occurs the forces of attraction between the chromosomes are presumed to overcome the repulsion between the pair of paired chromatids, and in cases where the somatic pairing is absent the forces of repulsion are supposed to exceed those of attraction.

More striking examples of somatic pairing have also been observed in doubled nuclei which have arisen through the failure of chromosomes to separate at a preceding division (e.g. *Sorghum*: Huskins and Smith, 1932; *Iberis*: Manton, 1935). Probably in these cases, the daughter chromosomes have remained together during the resting stage and therefore have been in a suitable position to pair.

The chromosome number of the species was confirmed in meiotic divisions. Twelve bivalents were noticed at diakinesis and metaphase I. Secondary association of bivalents was also noticed in polar view of metaphase I. The two divisions were quite regular and normal spores were generally formed.

One abnormality was, however, noticed in the material studied. In one anther locule several big pollen mother-cells were found intermingled with normal cells, the bigger cells containing double the number of chromosomes. Text-fig. 16 illustrates a portion of the anther locule showing two tetraploid cells and four diploid ones. Chromosomes in anaphase I in the normal and abnormal cells are illustrated in Text-figs. 17 and 18 respectively. A similar tetraploid pollen mother-cell was noticed by Verbrugge (1934) in a trisomic *Oenothera* mutant with fifteen chromosomes. These cells arose probably by duplication (i.e. autopolyploidy) in the last premeiotic telophase. This duplication may take place earlier in the premeiotic divisions, in which case groups of tetraploid cells result. Such cases are recorded in *Brassica* (Fukushima, 1931) and *Oenothera* (Cleland, 1929; Hakansson, 1926). These pollen mother-cells would give rise to gametes with diploid chromosome numbers, which are significant in connexion with the origin of polyploidy.

Crosses between *O. officinalis* and *O. sativa* succeed very rarely and the hybrid between them is highly sterile with practically no pairing of the parental chromosomes at meiosis (Ramanujam, unpublished, *b*). It is probable that structural differences of chromosomes are at least in part responsible for differentiation of the two species.

O. Barthii. Seeds of this species were obtained from Africa and plants grown at Regent's Park, London. This species is a perennial, propagated by rhizomes. The plants did not flower freely, so that material for study of meiosis could be obtained from only one panicle. Root tips were, however, fixed for study of somatic chromosomes (Text-fig. 19). The somatic number of chromosomes was determined to be twenty-four. The chromosomes are slightly smaller than those of *O. sativa*. The presence of satellited chromosomes could not be made out.

At diakinesis twelve bivalents were usually noticed (Text-fig. 20), though occasionally the chromosomes of one bivalent remained free. One bivalent was usually associated with the nucleolus at this stage. At metaphase I secondary association of chromosomes was frequently met with. The maximum association of five groups, consisting of three groups of two bivalents each, and two groups of three bivalents each, is illustrated in Text-fig. 21. The two divisions proceeded more or less normally in all the pollen mother-cells with the production of normal tetrads of spores. Three cells were, however, noticed in which at anaphase I delayed separation and consequent lagging of chromosomes on the plate was observed.

Crosses between this variety and *O. sativa* are not known.

O. longistaminata. This is also a perennial wild species, originally obtained from Senegal in west Africa. It resembles very closely *O. Barthii* in morphological characters and is probably a variety of the latter. The somatic chromosomes of this species were also determined as twenty-four (Text-fig. 22). The plant failed to flower, and hence no material for meiotic studies could be obtained.

O. latifolia. Seeds of this species obtained from Indiana, U.S.A., did not germinate and hence counts of chromosomes in the species could not be obtained. Gotoh and Okura (1933), however, report that this species, seeds of which were obtained from Cuba, had forty-eight chromosomes in root tips. Meiosis in the species has not been studied by them.

O. minuta. This species bears a close resemblance to *O. officinalis* in morphological characters and has a more or less similar distribution. The chromosome number of the species was recorded by Morinaga (1934) and Nandi (1936 *b*) to be $2n = 48$. Two varieties of this species obtained from Coimbatore and the Botanic Gardens, Buitenzorg, were examined for the somatic number of chromosomes, which was found to be forty-eight in each case. Text-fig. 23 illustrates somatic metaphase in one of the varieties.

At diakinesis in meiosis several cells examined showed twenty-four bivalents. Associations of more than two chromosomes were not observed either in diakinesis or metaphase I. Polar view of metaphase is illustrated in Text-fig. 24 and photomicrograph Plate II D. The chromosomes appear to be of two different sizes, being composed of twelve small bivalents and twelve big ones. This difference in size of the bivalents was also noticed by Nandi (1936 *b*). It is likely that *O. minuta* arose as an amphidiploid from a sterile diploid hybrid of *O. officinalis* and another species, probably *O. sativa*. As already mentioned, the hybrid between *O. officinalis* and *O. sativa* is completely sterile, with practically no pairing of parental chromosomes at meiosis. A duplication of chromosomes in the sterile hybrid may give rise to a fertile amphidiploid hybrid with $2n = 48$ chromosomes, resembling *O. minuta*.

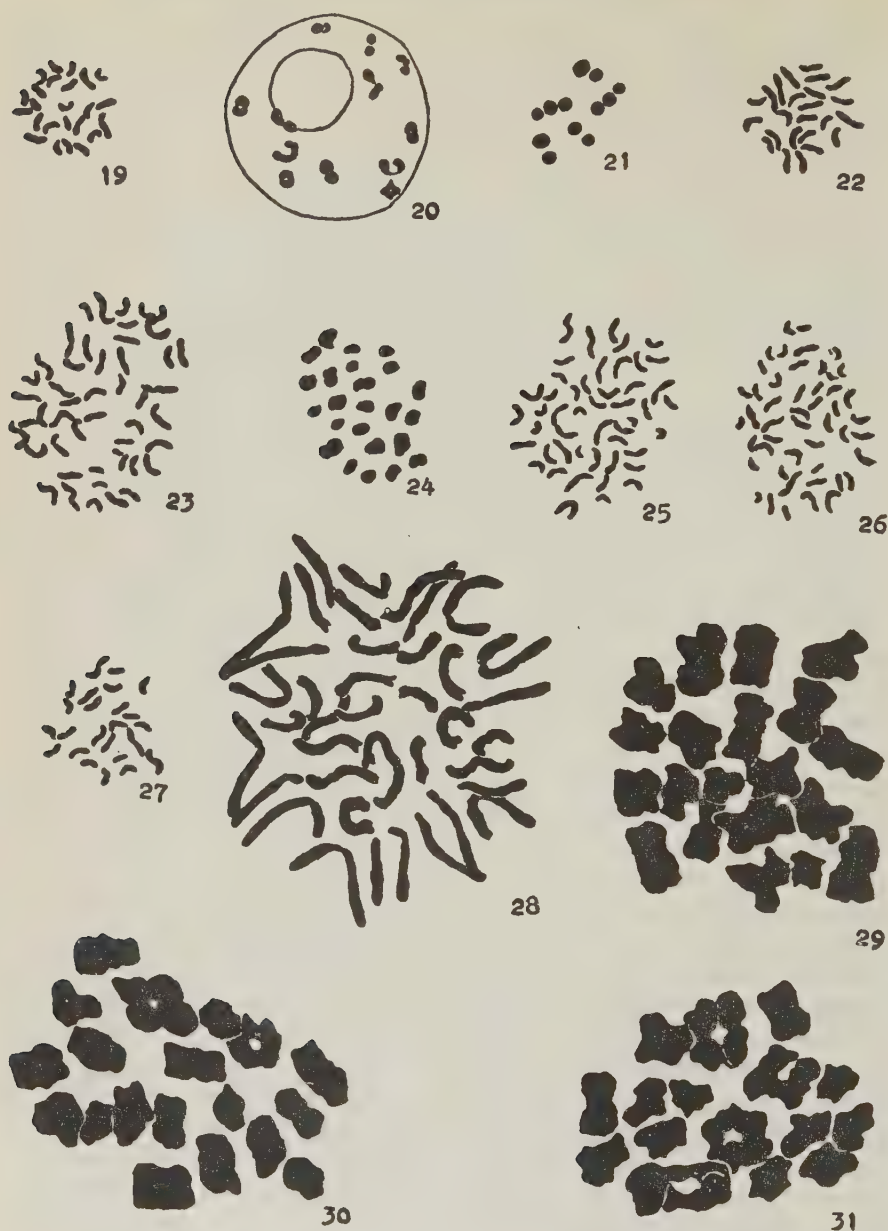
The genus Leersia. The species of *Leersia* are perennial marsh grasses widespread in tropical and temperate regions. The chromosome number of two species was determined for the first time from a study of somatic mitosis in them.

L. oryzoides was obtained from two sources, viz. America and England. Both materials showed $2n = 48$ (Text-fig. 25) chromosomes in root-tip mitosis. The chromosomes are small and more or less similar to those of *Oryza*. Seeds of *Leersia hexandra* were obtained from Indiana and the Philippine National Herbarium, and plants were grown at Regent's Park, London. Examination of root-tip mitosis in these plants showed forty-eight chromosomes in each case (Text-fig. 26), the chromosomes resembling those of *L. oryzoides*. The plants failed to flower in the greenhouse in London.

Hygroryza. Root tips of *Hygroryza aristata*, an aquatic grass found extensively in India, were examined for the first time for somatic chromosomes. The somatic number for the species was found to be twenty-four (Fig. 27).

Lygeae.

Lygeum spartum. This monotypic genus, originally included in the Oryzeae, has been removed from them by Hutchinson (1934) and put into a separate tribe by itself because of its different habitat and morphological characters.



TEXT-FIGS. 19-31. All drawings were made at bench level with the aid of a camera lucida. An apochromatic objective N.A. 1.3 was used in conjunction with Zeiss eyepiece K 30 giving an approximate magnification of 4,200 diameters. Fig. 19. *Oryza Barthii*. Somatic metaphase ($2n = 24$). Fig. 20. *Oryza Barthii*. Diakinesis with twelve bivalents. Fig. 21. *Oryza Barthii*. Polar view of metaphase I showing maximum secondary association, i.e. two groups of three bivalents each and three groups of two bivalents each. Fig. 22. *Oryza longistaminata*.

Avdulov (1931), however, has kept it in the tribe because of its similarity with the Oryzeae in its possession of compound starch grains and type II disposition of chlorophyll tissue (in type II the chlorophyll tissue occupies the entire area between the vascular bundles, and in type I it is restricted to two layers around the vascular bundles).

The somatic number of chromosomes for the species was determined from mitosis in root tips to be forty (Text-fig. 28). This would be an octoploid on the basis of five as basic number. The chromosomes are quite long compared to those of the other species in Oryzeae.

A preliminary examination of the meiosis in pollen mother-cells was made from material fixed in plants growing at Kew Gardens and the somatic number was confirmed. At diakinesis and metaphase I, twenty bivalents were noticed in certain pollen mother-cells (Text-fig. 29). In others, besides bivalents, quadrivalents ranging from one to four in each cell, were noticed. Text-figs. 30 and 31 illustrate metaphase I with one and four quadrivalents respectively. The quadrivalents appeared to be formed by both small and big chromosomes in the complement and fairly normal pollen was formed at the end of the two more or less normal divisions.

DISCUSSION

Classification of the tribe and species differentiation.

The classification of the tribe into (1) Zizaniinae, and (2) Oryzinae, adopted by Hutchinson (1934) is in the main confirmed by cytological results obtained so far. The section Zizaniinae, of which only one genus could be examined, is likely to be characterized by the basic number five. The two species of *Zizania* examined in this study with $2n = 30$ chromosomes each, are hexaploids on that basis. The section Oryzinae, of which representatives of all the three genera were examined, showed a basic number of twelve chromosomes. Two species of *Oryza*, viz. *O. minuta* and *O. latifolia*, are tetraploid and the others diploid; two species of *Leersia* are tetraploid and one species of *Hygroryza* is diploid. As already mentioned, the basic number twelve in *O. sativa* at least has been shown to be derived from an original basic number five, by secondary polyploidy (Nandi, 1936 b; Sakai, 1935). It is likely that both *Leersia* and *Hygroryza* also developed from this original basic number. Study of meiosis, including secondary pairing in these species, should throw light on

Somatic metaphase ($2n = 24$). Fig. 23. *Oryza minuta*. Somatic metaphase ($2n = 48$). Fig. 24. *Oryza minuta*. Metaphase I with twenty-four bivalents showing big and small bivalents (same as photomicrograph Plate II D). Fig. 25. *Leersia oryzoides*. Somatic metaphase ($2n = 48$). Fig. 26. *Leersia hexandra*. Somatic metaphase ($2n = 48$). Fig. 26. *Hygroryza aristata*. Somatic metaphase ($2n = 24$). Fig. 28. *Lygeum spartum*. Somatic metaphase ($2n = 40$). Fig. 29. *Lygeum spartum*. Polar view of metaphase I with twenty bivalents. Fig. 30. *Lygeum spartum*. Polar view of metaphase I with eighteen bivalents and one quadrivalent. Fig. 31. *Lygeum spartum*. Polar view of metaphase I with twelve bivalents and four quadrivalents.

this point. It is therefore possible that the tribe Oryzeae developed from an original ancestor with five as its basic chromosome number. While one section, the Zizaniinae, retained its original basic number and evolved a polyploid series, the other section, Oryzinae, developed a secondary basic number 12, through secondary polyploidy and developed a polyploid series on the basis of this new number.

The position of *Lygeum* in the tribe has remained doubtful, owing to its different habitat and morphological characters. Cytologically, *Lygeum spartum*, with $2n = 40$ chromosomes is an octoploid on the five basis and may be included in the Zizaniinae section of the Oryzeae. But the size of its chromosomes is bigger than any of the Oryzeae. The size of the chromosomes by itself may not provide sufficient grounds for separating *Lygeum* from the Oryzeae in view of the fact that genotypically controlled differences in size of chromosomes are known to characterize many related varieties and species. In the Andropogoneae three species, viz. *Sorghum versicolor*, *S. purpurea sereceum*, and *S. dimidiatum*, have been found to have much larger chromosomes than other species to which they are closely related systematically (Hill, 1934). In the Oryzeae itself, such differences in size of somatic complements are noticed, e.g. the chromosomes of *Zizania* are slightly larger than those of *Oryza*. There is also evidence that *O. officinalis* has larger chromosomes than *O. sativa* (Ramanujam, unpublished, b). In several families of dicotyledons (e.g. *Tradescantia*e, Anderson, and Sax, 1936) large variations in size of somatic complements are observed in related species. It is therefore suggested that in view of its large chromosomes coupled with its different habitat and morphological characters, *Lygeum* should be put in a separate section in the Oryzeae.

As regards the factors involved in the differentiation of species in the tribe, more evidence should come from the study of interspecific and intergeneric crosses. The results of this study, however, indicate a few conclusions on this subject. The existence of chromosome numbers in multiples in the different genera indicates that polyploidy has played an important part in evolution. As already mentioned, *O. minuta* with $2n = 48$ chromosomes may have originated as an allotetraploid from a cross between two species of *Oryza* with $n = 12$ chromosomes each, one of them being *O. officinalis*. The absence of quadrivalents and the presence of twelve small bivalents and twelve large bivalents at meiosis lend additional support to this view. It is also likely that tetraploid species of *Leersia* originated through allopolyploidy. This, however, requires to be confirmed by study of meiosis in these plants. *Lygeum spartum*, with $2n = 40$ chromosomes, shows quadrivalents at meiosis, ranging from one to four in each cell. There is evidence that these quadrivalents are formed by both small and large chromosomes. The formation of these quadrivalents may be due either to segmental interchange between non-homologous chromosomes or to autopolyploidy. The presence of four more or less similar sets of chromosomes in the somatic complement, together with

the high percentage of normal pollen formed in the plant, suggest that *Lygeum spartum* may be an autopolyploid.

In the genus *Oryza* itself, structural and genic differences may have been responsible for differentiation of species. *O. sativa* crosses readily with *O. glaberrima*, giving fertile hybrids which show normal segregation of characters in the progeny. The hybrid between *O. officinalis* and *O. sativa* is obtained with great difficulty and is sterile, with no pairing between parental chromosomes at meiosis. While genic differences alone may distinguish the two species in the former case, perhaps structural differences are involved in the latter.

Position of the tribe in the family.

It is too early to say anything definite on this point until more genera are examined cytologically. This study has, however, shown that the original basic number for the tribe is five and not twelve, as was supposed by Avdulov. On the presumption that the basic number for the tribe was twelve, he thought the Oryzeae did not belong to the Panicoideae, although the chromosomes were small and of the panicoid type. He therefore included them in a special group with small chromosomes in multiples of twelve, in the sub-family Poateae Hitchcock corresponding to Pooideae Brown. With the establishment of the basic number for the Oryzeae to be five, their closer relationship to Panicoideae than to Pooideae becomes evident. Several species in the tribe Andropogoneae of the sub-family Panicoideae have haploid chromosome numbers of five and multiples of it. As regards some of the morphological characters, such as the reduction of the florets and suppression of the glumes, the Oryzeae suggest the Panicoideae, and for this reason they were originally included in this sub-family by systematists. Besides, some of the members of the Oryzeae, viz. *Oryza* and *Lygeum*, have bicellular hairs in the epidermis, which Avdulov thinks is a characteristic of the Panicoideae. The only characters in which the Oryzeae differ from the Panicoideae are the possession of type II leaf and compound starch grains, which are important characters of the Pooideae. When all these facts are considered it may not be far wrong to conclude that the Oryzeae are more nearly related to Panicoideae than the Pooideae.

SUMMARY

Chromosome studies, with a view to ascertaining taxonomical relationships in the tribe, were undertaken for the first time and results from several species in five genera lead to the following conclusions.

The basic number of chromosomes for the tribe is five and not twelve, as was supposed by Avdulov.

While one section, the Zizaniinae, retained the original basic number and developed a polyploid series, the other section developed a secondary basic number twelve, through secondary polyploidy and built up a polyploid series

on this new number. Thus the taxonomical classification of the tribe into two sections is supported by cytological observations.

It is suggested that *Lygeum* with large chromosomes and different habitat, with $2n = 40$ chromosomes, be placed in a third section.

A brief discussion regarding species differentiation in the tribe and their position in the family is included.

ACKNOWLEDGEMENTS

I have great pleasure in acknowledging my indebtedness to Professor R. R. Gates, and desire to express my grateful thanks for his kindly interest and helpful criticism during this investigation.

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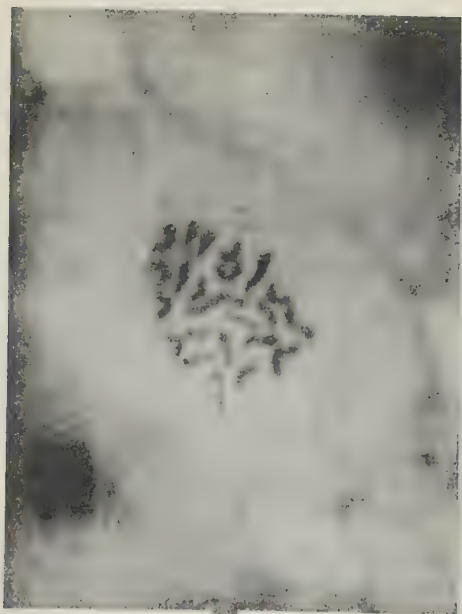
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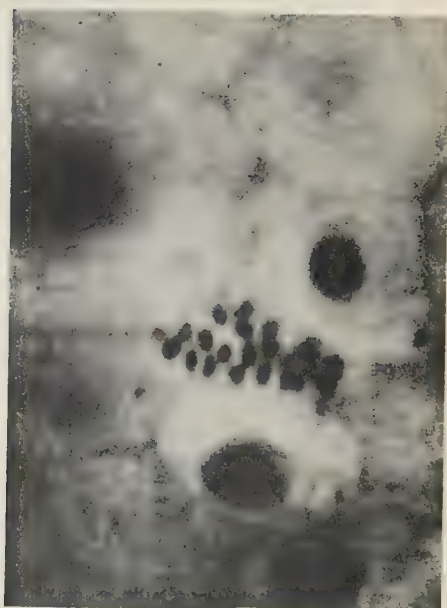
EXPLANATION OF PLATE II

Illustrating Dr. S. Ramanujam's paper on 'Cytogenetical Studies in the Oryzeae. I. Chromosome Studies in the Oryzeae'.

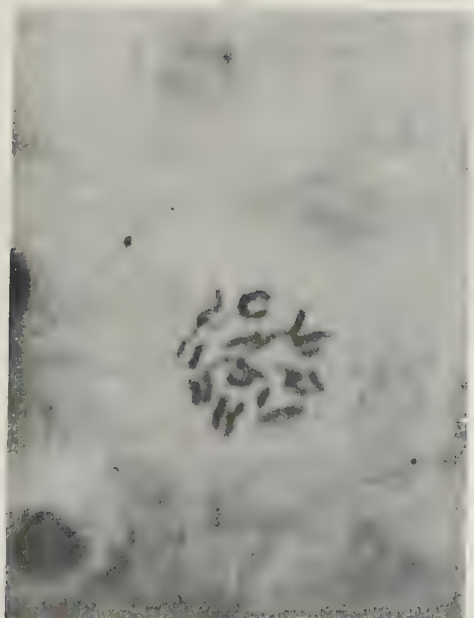
- A. Somatic metaphase in the variety T 24 with normal chromosomes ($2n = 24$).
- B. Somatic metaphase in the variety T 24 with highly condensed chromosomes ($2n = 24$).
- C. Mitotic metaphase in *Oryza officinalis* showing somatic pairing of chromosomes.
- D. Polar view of metaphase I in a pollen mother-cell of *Oryza minuta*. Small and big bivalents are noticed.



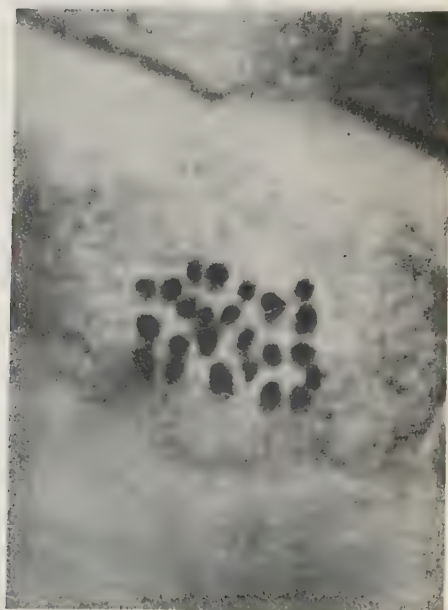
A



B



C



D

The Monocotylous Seedlings of Certain Dicotyledons. With Special Reference to the Gesneriaceae

BY

ARTHUR W. HILL

With Plates III to V and twenty-three Figures in the Text

CERTAIN dicotyledonous plants, as is well known, normally develop only a single cotyledon on the seedling instead of the typical pair, and all such cases offer interesting fields for study and speculation.

Very frequently the loss or suppression of one of the cotyledons is associated with the development of a bulbous habit in a particular genus or species. *Cyclamen* affords a good example and here, as has been shown (Hill, 1920), the second cotyledon is actually present but, with very rare exceptions, it remains rudimentary and suppressed.

The late Miss Ethel Sargent (1903, p. 76) overlooked this rudiment and stated that the anatomical structure of the *Cyclamen* cotyledon 'suggests very strongly that the cotyledonary member consists of two seed-leaves united into a solid tube'. Since the cotyledon of *Cyclamen* is undoubtedly a single structure and not two united cotyledons, as she suggested, one is tempted to feel a little sceptical as to the value of the anatomical evidence for fused cotyledons which has been brought forward in the case of some of the other monocotylous dicotyledons.

Ranunculus Ficaria and the bulbous Umbellifers (*Bunium* and *Conopodium*) are cases in point. These seedlings normally show no trace of a second cotyledon and though the single cotyledon of *Bunium* has been repeatedly amputated, as has been done with *Cyclamen* and some other seedlings, no indication of a second cotyledon has been discovered. Here also it has been argued (Sargent, 1903, p. 63) that the seed-leaf of *Ranunculus Ficaria* represents two fused cotyledons, but Metcalfe (1936), in his recent careful studies of the seedlings and their anatomy carried out in the Jodrell Laboratory at Kew, has shown conclusively that the single-leaf of *R. Ficaria* does not represent a double structure.¹

In *R. Ficaria*, therefore, it seems clear that one cotyledon has been wholly suppressed, as also appears to be the case in *Bunium elegans*.

No trace of a second cotyledon can be detected in *Bunium*; and when the anatomical structure of the single strap-like cotyledon is examined, it is

¹ Seedlings of *R. Ficaria* very occasionally possess two cotyledons, and in normal seedlings the second cotyledon is present as a parenchymatous protuberance which fails to develop.

exactly comparable, as regards its vascular bundles, with the structure of one of the two cotyledons of the allied *Anethum graveolens*.

There is nothing to suggest that this single seed-leaf is in any way abnormal as regards its vascular structure; it seems, therefore, safe to assume that only one cotyledon is present in the bulb-bearing species of the genus *Bunium*, the other having been entirely lost.

Another case of abnormal dicotyledonous seedlings is afforded by the bulbous and tuberous species of *Peperomia* (Hill, 1906) from the High Andes of Bolivia and Peru, and from the mountains of Mexico, but here the two cotyledons are present, each performing a separate function, one emerging from the seed to become the assimilating leaf, while the other never leaves the seed but functions wholly as an absorbent organ; the seedling thus having a falsely monocotyledonous appearance on casual examination.

Miss Sargent (1903, pp. 76, 77) gives a list of twelve monocotylous dicotyledons belonging to five natural families (Fumariaceae, Umbelliferae, Primulaceae, Lentibulariaceae and Nyctaginaceae). One of these twelve, *Cyclamen*, must now be excluded from the list; the three species of *Abronia* (Nyctaginaceae), which she considered doubtful, should also be omitted, since it seems generally agreed that both the cotyledons are present, one developing later than the other. It is of interest to note in this connexion that the seedlings of the closely allied genus *Allionia* have one of the cotyledons in the embryo only about half the size of the other.

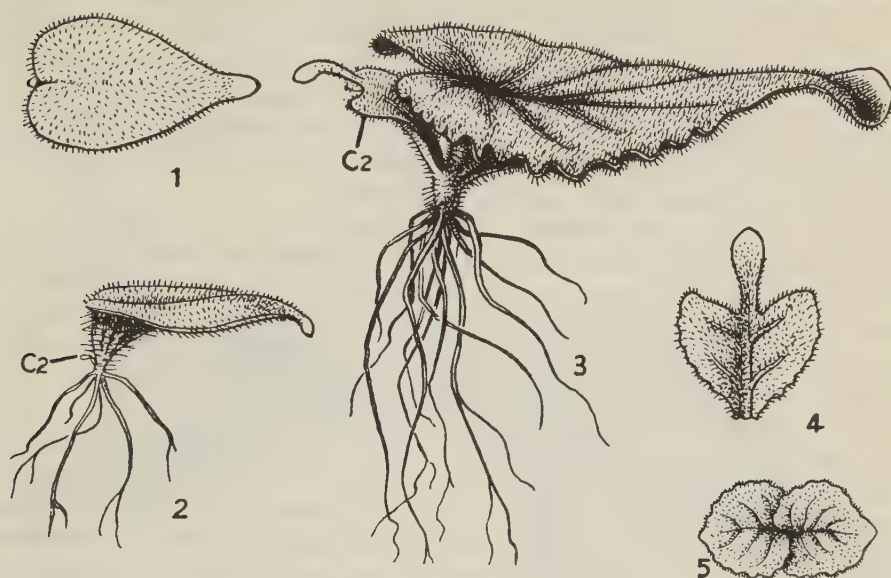
With regard to the abnormal Fumariaceae and Umbelliferae, I feel we are on safer ground in following Hegelmaier, Irmisch, and Schmid, who regarded the cotyledon as a single organ, the second one having been almost wholly or entirely suppressed. Hegelmaier (1878), for instance, found that *Bunium Bulbocastanum* possessed the rudiments of a second cotyledon and he also met with an embryo with both cotyledons developed. He also found rudiments of a second cotyledon in *Erigenia bulbosa* (Umbelliferae) and *Claytonia virginica* (Portulacaceae), both of which, according to Holm (1901), possess only a single seed-leaf. The second cotyledon of *Corydalis cava* is considered by Schmid (1902) to be represented by a protuberance, and this is confirmed by Coulter and Chamberlain (1904, p. 206). Miss Sargent, however (1903, p. 69), apparently misquotes Schmid in saying 'we know from the researches of Dr. Schmid that no traces of the original bicotyledonary structure are to be found in the early history of the embryo of *Corydalis cava*'.

Other species of *Corydalis*, for example, *C. nobilis* and *C. lutea*, have normal seedlings with two fully developed cotyledons; thus in *C. cava* suppression of one cotyledon rather than fusion seems, both on Schmid's evidence and also on general grounds, to be the more probable explanation.

Then there are species of *Pinguicula* which apparently possess only a single cotyledon. Seeds and seedlings of *P. vulgaris* have been examined by Dr. Metcalfe in the Jodrell Laboratory, and it seems probable that in this case also one of the cotyledons has aborted. The deeply bifid cotyledon of

P. lusitanica, giving the appearance of two cotyledons, is morphologically equivalent to the single one of *P. vulgaris*.

Trapa natans (Onagraceae) again is another abnormal dicotyledon as regards its seedling, for, although it starts life with two cotyledons of equal size,



TEXT-FIGS. 1-5. Figs. 1 and 2. *Streptocarpus Galpinii*, seedlings in surface and side view; the densely hairy adventitious lamina of the persistent 'cotyledon' carries the glabrous original cotyledon at its apex; note the minute glabrous second cotyledon (C. 2) ($\times 3$). Fig. 3. *Streptocarpus* sp., a seedling showing the development of an adventitious lamina to the second cotyledon (C. 2), the actual cotyledon remaining a distinct entity ($\times 3$). Fig. 4. The same, the second cotyledon in surface view ($\times 3$). Cf. Plate V, Figs. 10, 11. Fig. 5. The same, a seedling with both cotyledons developing ($\times \frac{1}{2}$).

one remains quite small and stops growing, while the other grows on and pushes the young plant out of the seed, at the same time acting as an absorbent organ.

It is, however, with certain remarkable genera of Gesneriaceae, belonging to the tribe Cyrtandreae and mostly to the sub-tribe Didymocarpeae, especially *Streptocarpus*, *Boea*, *Didymocarpus*, *Chirita*, *Klugia*, *Saintpaulia*, *Haberlea*, and also *Moultonia*, *Monophyllaea*, and *Didissandra*, and some other genera, that this paper is particularly concerned.

In *Streptocarpus* the seedlings in the earliest stages possess two equal cotyledons and the seedlings at first are typical of those of normal dicotyledons. In the course of a few days, however, one of the cotyledons increases considerably in size, while the other remains stationary and makes no further growth; eventually it turns yellow and dies off (Crocker, 1860).¹ In the case

¹ Seedlings of *Streptocarpus polyanthus*, *S. Rexii*, and *S. biflorus* are described and figured. The figures of *S. biflorus* indicate the absence of hairs on the second cotyledon but there is no reference to this point in the text. Seedlings of *Chirita Moomii* with unequal cotyledons are also mentioned.

of the well-known species of *Streptocarpus* which possess only one leaf, *S. Dunnii* (Pl. V, Fig. 11), *S. Wendlandii*, *S. polyanthus*, &c., the cotyledon which persists becomes the leaf of the plant, and in these species no other leaves are developed¹ but the plants consist only of a single cotyledon, which becomes greatly enlarged by basal intercalary growth and develops into a large leaf without a stalk. This state of affairs is typical of what may be termed the cotyledonary (or unifoliate) series of these somewhat aberrant Gesneriaceae, and the series includes also the genera *Platystemma*, *Moultonia*, *Monophyllaea*, *Trachystigma*, and *Acanthonema*, and the following species, *Didisandra sesquifolia*, *Didymocarpus pygmaea*, *Chirita monophylla*, *C. bifolia*, and sometimes *C. hamosa* and *C. capitis* (see below).

Other species of *Streptocarpus*, however, such as *S. Rexii* and *S. parviflorus*, develop a rosette of three or four or more leaves, and have been placed for convenience in the group or series *Rosulatae*. In these species one cotyledon is retained, as in the unifoliate group, and is usually the largest leaf of the rosette. Other genera which can conveniently be placed in this group are *Saintpaulia*, *Ramondia*, *Haberlea*, *Conandron*, *Tremacron*, *Oreocharis*, and possibly *Boea*; *Chirita Trailliana* should also be included.

In addition to these two groups there is a third consisting of the caulescent species of *Streptocarpus* and *Chirita*—some of which, for example *C. hamosa* and *C. capitis*, may be unifoliate under certain conditions (Pl. IV, Figs. 6–8); this group may be termed *Caulescentes*. *Briggsia aurea* and allied species, and the genera *Epithema*, *Klugia*, *Rhynchoglossum*, *Leptoboaea*, and *Ornithoboaea* should be included in this group.

In these cases the adult plants are herbaceous since the plumule develops and bears erect stems with numerous opposite leaves (except in *Klugia* and *Rhynchoglossum* where they are alternate), but, though they are so dissimilar in habit to the unifoliate species, the seedling structure is as far as is known identical, only one of the two cotyledons developing, while the other aborts at an early stage (Dickson, 1883)². The species of *Streptocarpus* which have principally been studied shows this peculiarity in their seedlings whether they occur in Siam and Burma or whether, as in the majority of the species, they are natives of Africa.

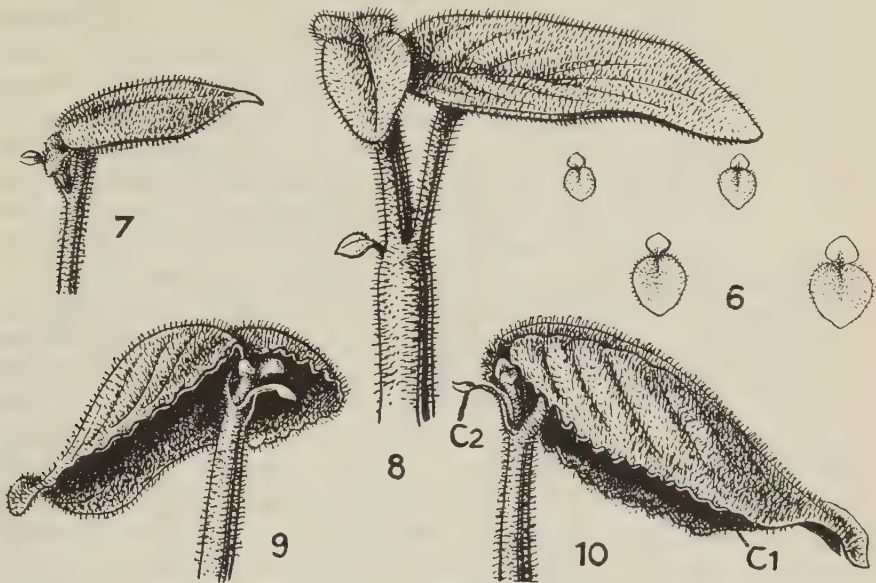
Dr. Metcalfe, who has kindly examined numerous seedlings of *Streptocarpus* informs me that no trace of a plumular bud can be found in the seedlings of the monophyllous species. The base of the developing cotyledon becomes circular in cross-section and occupies all the available space in which a normal plumular bud could arise. The cells at the base of the lamina and some of those of the midrib of the cotyledon remain meristematic and through

¹ Both cotyledons occasionally develop in *S. Dunnii* and rarely a second small leaf, apparently adventitious, is produced in some of the other species.

² The plate (*Streptocarpus caulescens*) is of interest since the seedling closely resembles that of *Chirita* with one large petioled cotyledon bearing a shoot in its axil. The second, undeveloped cotyledon, is at a lower level and has a suppressed axillary bud.

their activity the cotyledon increases in length. It is also apparently in association with this same meristem that the reduced shoot-system ultimately arises.

The genus *Chirita* offers a close parallel to *Streptocarpus* in having only one persistent cotyledon, the other ceasing to grow and often withering a few



TEXT-FIGS. 6-10. Fig. 6. *Streptocarpus caulescens*, young seedlings showing the developing cotyledon hairy with a glabrous tip and the arrested one glabrous ($\times 3$). Fig. 7. The same, an older seedling, the persistent 'cotyledon' has developed a petiole ($\times 3$). Fig. 8. A caulescent species of *Streptocarpus* from East Africa which has developed a petiole to the persistent cotyledon and exactly resembles the seedlings of *Chirita lavandulacea* ($\times 3$). Figs. 9 and 10. Seedlings of *Chirita lavandulacea* to show the large petioled 'cotyledon' (C. 1) and the small glabrous one (C. 2) ($\times \frac{3}{4}$).

days after it has emerged from the seed.¹ Both in the herbaceous *Streptocarpus* and in *Chirita* it is of interest to find that it is only in the axil of the persistent cotyledon that functional axillary buds are developed (see Text-figs. 6-10, 20-23, and Pl. III, Figs. 1 and 2), and this axillary bud, which in *Chirita* is often accompanied by an extra bud, develops strongly and makes the young plant very one-sided with its single, well-developed, leaf-like cotyledon and the strong axillary shoot, which may often be nearly as stout as the main axis.

There is one interesting point of difference between the caulescent species of *Streptocarpus* and *Chirita*. In the former the flowers are borne on axillary

¹ *Chirita capitis*, *involucrata*, *lavandulacea*, *Blumei*, *pumila*, and *rupestris* agree closely with *Streptocarpus* in having only one cotyledon developed, the other remaining stationary after it has grown for a few days.

In *C. acuminata* and *C. barbata* both cotyledons develop, but in *C. acuminata* one is larger than the other.

racemes or panicles, an extra-axillary bud being also usually present, while in *Chirita*¹ the flowers, in certain species, are partly axillary and partly are borne on the upper-leaf petioles and midrib of the leaf in acropetal succession as in the unifoliate and rosulate *Streptocarpus* (Pl. III, Figs. 2, 3, 4).

The genus *Chirita* affords another close parallel to *Streptocarpus* since a few species (such as *C. bifolia* D. Don, *C. monophylla* C. B. Clarke, *C. hamosa* R.Br. var. *unifolia* C. B. Clarke, *C. viola* Ridl., and *C. pumila* D. Don) are normally monophyllous throughout their life-history and bear their flowers at the base and on the midrib of the single leaf, which is the enlarged cotyledon (Pl. IV, Figs. 5, 6, and 8). In some cases a second small leaf may be present as in *C. bifolia*, which probably represents the second cotyledon.² There are also a few rosulate species, *C. Trailliana* and allies, while other species tend to be dimorphic in their adult stages, being either monophyllous or herbaceous according to the conditions under which they may be growing.

Specimens of *Chirita capitis* Craib, for instance, which Dr. A. F. G. Kerr found growing on limestone rock (see Pl. IV, Figs. 5 and 6) at Petchaburi, Siam, and also on an old plastered wall at Bangkok, are purely monophyllous, bearing flowers on the midrib of the lamina which has developed at the base of the cotyledon; such plants to all intents and purposes are morphologically identical with the monophyllous species of *Streptocarpus* or with *Moultonia* or *Monophyllaea*. In such conditions the plumule apparently is suppressed, but where plants are grown under better conditions, the plumule develops and specimens 1–2 ft. or more in height, bearing pairs of leaves above the cotyledon, may be found (Pl. IV, Fig. 7). These tall plants, however, are always one-sided at the base, since only one of the two cotyledons—or rather the secondarily developed lamina at its base—persists as the lowest leaf, and in its axil an axillary shoot may also be produced of almost equal vigour to the main stem (Pl. III, Figs. 1 and 2; Pl. IV, Fig. 7)³. Other species of *Chirita* show similar cases of dimorphism, especially *C. viola* Ridl. Numerous specimens of this species collected by M. R. Henderson at Selat Panchor are, with one exception, unifoliate, while others collected at Bata Ayam bear stems over a foot high with four or five pairs of leaves above the cotyledonary leaf. *C. hamosa* R. Br. exhibits a similar state of affairs and C. B. Clarke gave the varietal name *unifolia* to the unifoliate specimens, collected on rocks on the Khasia Hills (Pl. IV, Fig. 8). *C. hamosa* normally is a plant some 2 ft. tall, with paired upper leaves, the flowers being borne in acropetal succession on their petioles. Plants of this species collected by A. Henry in Yunnan are of both unifoliate and herbaceous types. *C. bifolia* D. Don, from Simla and the

¹ The genus needs revision as it contains two or more well-marked types, differing widely in their habit-characters.

² To be certain of this point it will be necessary to study the development of the seedlings, whenever seed may be available.

³ Occasionally a rudimentary axillary bud may be seen in the axil of the second cotyledon both in *Streptocarpus* and *Chirita* but it never appears to develop unless the plumule and bud in the persistent cotyledon axil be damaged.

Siwaliks, is either completely unifoliate or, in some cases, a second quite small leaf develops opposite the persistent and enlarged cotyledon. This may either be the second cotyledon or a small plumular leaf, but from dried specimens it cannot definitely be determined. *Didissandra sesquifolia* C. B. Clarke, affords a closely parallel example. One other Chirita, *C. oculata* Craib, found by Dr. Kerr on limestone rocks at Kao Sakan, Siam, may be either unifoliate or herbaceous, depending no doubt on the habitat as in the case of *C. capitis*.

In the case of the unifoliate and rosulate *Streptocarpus*, and probably in the other unifoliate genera also, no trace of an apical or plumular bud can be found between the cotyledons such as occurs in a normal dicotyledonous seedling. The base of the persistent cotyledon, however, becomes circular in cross-section and takes the place which a normal plumular bud would occupy. The great development of the single leaf from the persistent cotyledon is due to the meristematic activity of the cells at the base of the lamina and the midrib of the cotyledon. From the activity of these cells arises the single 'leaf' of the unifoliate and acaulescent species of *Streptocarpus* which carries the cotyledon at its apex. The single adult leaf may, according to the views of some morphologists, be regarded as an 'adventitious' organ, since it arises directly from a meristem at the base of the cotyledon, the cotyledon itself remaining an entity, frequently clearly recognizable, at the tip of the adventitiously produced lamina.

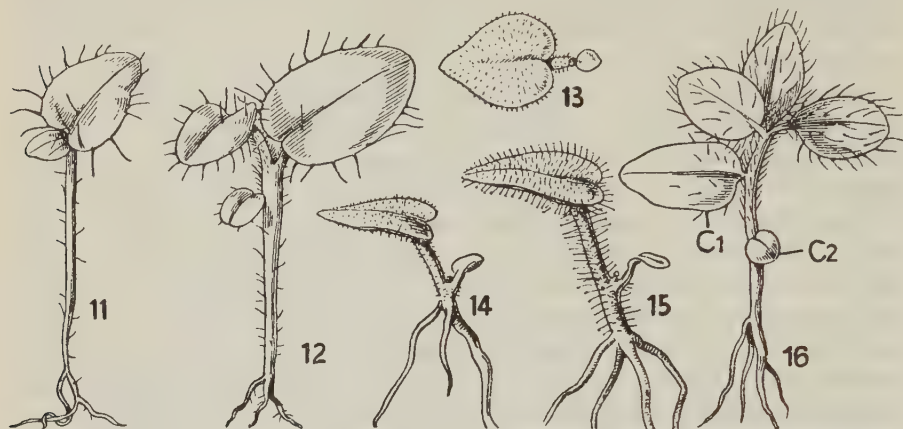
The meristem, however, which gives rise to this great laminal development is not a secondary but a primary meristem; hence, according to Goebel (1900, i, p. 42), it should not properly be regarded as an adventitious structure. This same meristem, which perhaps may be most usefully regarded as the delayed plumular bud, can give rise not only to the leafy tissue intercalated between the actual cotyledon and the hypocotyl, but also to the reduced shoot system consisting of the flowering shoots and their bracts and also, in the rosulate species, to the additional leaves which form the rosette, each of which closely resembles the cotyledonary leaf.

This meristematic tract, which has been referred to as a delayed plumular bud, may in favourable conditions develop normally and produce the shoot typical of the caulescent species of *Streptocarpus*, *Chirita*, and other caulescent genera. Despite the fact that it may not be strictly correct to consider the leaf of the unifoliate *Streptocarpus* as an adventitious organ, this secondarily produced lamina may well be regarded as an adventitious structure, since it is produced *de novo* from the meristematic cells at the base of the cotyledon, and is therefore a somewhat abnormal 'leaf' in its mode of origin.

Unfortunately it has not been possible to study the seedlings of all the genera to which reference has been made, but in addition to those of numerous species of *Streptocarpus* and *Chirita* the following deserve special mention.

Didymocarpus. The seedlings of the following four species of *Didymocarpus*, *D. crinita*, *D. platypus*, *D. paniculata*, and *D. Mortoni*, are for the first few days after germination exactly like those of a normal dicotyledon. Then,

as in *Streptocarpus* and *Chirita*, one increases in size while the other remains stationary. As development proceeds, however, the two cotyledons become separated, the enlarged persistent one being at a higher level than the other (Text-figs. 11–16 and Pl. V, Fig. 12). The intercalated tissue separating the



TEXT-FIGS. 11–16. Fig. 11. *Didymocarpus puncticulatus*, a young seedling showing the unequal cotyledons at the same level ($\times 6$). Fig. 12. The same, an older stage, the persistent cotyledon (C. 1) has been carried up by the development of the plumule ($\times 6$). Fig. 13. *Didymocarpus Mortoni*, a seedling from above showing the small glabrous second cotyledon and the large hairy persistent one ($\times 9$). Figs. 14 and 15. The same, later stages showing the development of a petiole to the persistent 'cotyledon' ($\times 9$). Fig. 16. *Didymocarpus platypus*, a well-advanced seedling showing the persistent cotyledon (C. 1) at a higher level than the small one (C. 2) ($\times 4$).

two cotyledons is due to the meristematic activity of the cells at the base of the enlarging cotyledon and may be regarded as an elongation of the petiole of the cotyledon or of the 'delayed plumular axis'.¹

This displacement of the persistent cotyledon, which also takes place in some species of *Chirita*, is found in both the acaulescent and caulescent species of *Didymocarpus*.

Seedlings of an unnamed species of *Didymocarpus* raised from seed sent from Malaya show both cotyledons almost equal in size, though one of them is usually rather larger than the other. They differ also from those of the other species mentioned above in being both at the same level.²

Klugia. This genus, which occurs both in Tropical Asia and America, agrees with *Streptocarpus*, &c., in the anisophylly of its cotyledons. According to Fritsch (1904, p. 46) the cotyledons are normal and equal in size in the early stages of germination, but in the course of a few days one ceases to grow

¹ This region is called the 'mesocotyl' by Fritsch (1904).

² Of the 58 species of *Didymocarpus* referred to by C. B. Clarke (1883, p. 70) only one, *D. pygmaea* Clarke is monophyllous. Some species, of which seeds have germinated at Kew, have normal seedlings, with both cotyledons more or less equal in size. It seems possible that a careful study of the genus and of the seedlings of the various species might result in a subdivision of this somewhat diverse and widespread genus.

and the other, which is carried to a higher level as in *Didymocarpus*, increases in size and becomes a foliage leaf similar to those subsequently developed on the stem as in the caulescent species of *Chirita*.

Seedlings of *Klugia Notoniana* and *K. zeylanica* raised at Kew showed well-marked anisophylly of the cotyledons, exactly as in *Streptocarpus*. *Klugia*, however, differs from *Chirita* and *Streptocarpus* in having the plumular leaves alternate instead of being opposite.

Monophyllaea Horsfieldii (Ridley, 1906), from Malaya, which possesses only a single leaf throughout its life-history, is another striking example of anisophylly, the second smaller cotyledon dying off at an early stage in the life-history of the seedling, while the other develops into the single leaf of the adult plant by intercalary meristematic activity at its base.

Other genera and species possessing unequal cotyledons are *Loxocarpus* (*L. Holttumii*),¹ *Didissandra* (*D. atrocyanea*),¹ and *Stauranthera grandiflora*.²

Moultonia (Balfour & Smith, 1915), from Sarawak, and *Acanthonema* (Hooker, 1862), from West Africa, probably have similar anisophyllous seedlings, judging from the *Streptocarpus*-like adult unifoliate plants, but unfortunately the seedlings have never been described.

Among rosulate genera *Saintpaulia* (Hooker, 1895), *Ramondia* (Skan, 1918), *Haberlea* (Hooker, 1882) and *Oreocharis* (*O. primuloides*) have seedlings with unequal cotyledons, though less markedly so than in *Streptocarpus*. Those of *Saintpaulia* are of interest since the second cotyledon in many cases is more or less glabrous—the larger one developing numerous hairs as it increases in size (Text-figs. 17–18).

Haberlea has one of its two cotyledons slightly larger than the other, but both turn brown and fall off, the smaller one, however, is the first to die while the larger one remains green for some time; both are slightly fleshy when green and are also glabrous. The larger one is from 1 to 1.25 mm. in length, and the smaller from 0.85 to 0.95 mm. long.

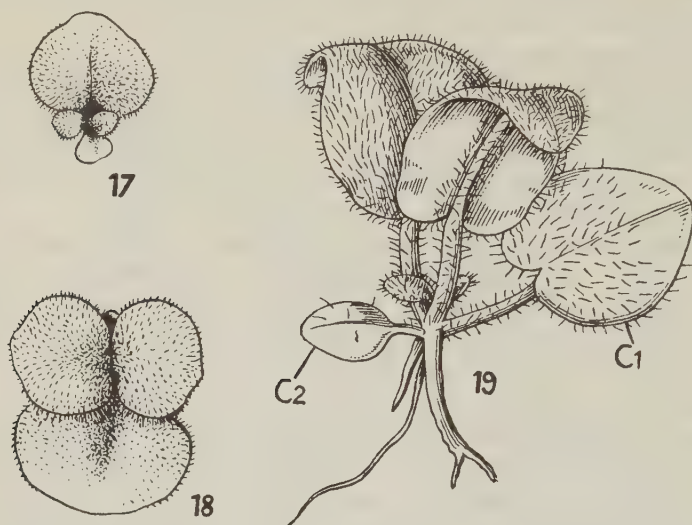
Oreocharis primuloides, the Japanese species, is another plant which has a rosette of leaves in an adult stage and shows unequal cotyledons in the seedling. In this example (Text-fig. 19) the tip of the enlarged cotyledon is glabrous, but the base hairy as are the normal foliage leaves. At the stage illustrated the large cotyledon was 2.5 cm. long and its petiole 1.75 cm., the small one 1.5 mm. long and its petiole 0.5 mm. It is not yet certain whether the larger one may persist, as one of the rosette-leaves.

Chirita lavandulacea (Stapf, 1925), the species most commonly grown in this country, is worthy of careful study in attempting to trace the relation of the one-leaved species of *Streptocarpus*, *Chirita*, *Monophyllaea*, &c., to those which have developed the herbaceous habit. It will be noticed (Text-figs.

¹ Seedlings of these two species were kindly sent to Kew by Mr. R. E. Holttum, Director of the Botanic Gardens, Singapore.

² Drawings and notes by the late Mr. James Fraser, made when working for Lord Avebury on his book on seedlings.

20-23 and Pl. III, Figs. 1 and 2) that the cotyledon and the lower foliage leaves in *C. lavandulacea* have fairly long petioles, but when the plant reaches the flowering condition the upper floriferous leaves are almost or completely sessile. Moreover, it will be seen, more especially where the leaves are quite



TEXT-FIGS. 17-19. Figs. 17 and 18. Seedlings of *Saintpaulia ionantha*; the smaller cotyledon is almost glabrous, while the larger, like the plumular leaves, is very hairy ($\times 3$). Fig. 19. *Oreocharis primuloides* seedling showing the unequal cotyledons (C. 1, C. 2); the larger has developed a long hairy petiole, the lower portion only of the blade is hairy, and in this respect resembles the plumular leaves ($\times 9$).

sessile, that the flowers, which at first are axillary, are produced, by a proliferation of the midrib of the leaf, exactly as are the flower buds along the cotyledonary midrib in the monophyllous species of *Streptocarpus*.¹ If, therefore, one of these flower-bearing leaves of *Chirita* be severed from the stem, the unit is to all intents and purposes a unifoliate *Streptocarpus*, and it may—as I think—be legitimately suggested that when a herbaceous *Chirita* reaches the flowering stage it exhibits a reversion to an earlier or ancestral condition, showing that the herbaceous character in this genus, and also in *Streptocarpus* and the other allied caulescent genera, has developed from a monophyllous condition which should be regarded as the more primitive.

In making an assumption of this nature, which is strengthened by several facts not otherwise readily explicable, the anisophylly of the cotyledons in the herbaceous species is given a plausible explanation. The 'reversion' to monophylly in the species of *Chirita* which have been mentioned—especially

¹ It should be mentioned that the caulescent species of *Streptocarpus* always bear their inflorescences in the leaf axils and never produce flower buds on the leaf petioles or midribs as in *Chirita lavandulacea* and other species.

C. capitis and *C. oculata*—when grown on rocks or other unfavourable media, may, I consider, be held to give definite support to this suggestion.

On this assumption, then, one may presume that when the monophyllous character was developed in primitive *Streptocarpus*, *Monophyllaea*, *Didymocarpus*, *Chirita*, &c., owing to the habitats they selected, one leaf only—one of the two cotyledons—proved to be sufficient for the life-cycle; the second cotyledon, being then superfluous, ceased to develop at an early stage in the life of the seedling. The second cotyledon, therefore, having ceased to function, did not regain its vigour when later these genera—which may all have evolved from a more primitive monophyllous type—developed in certain species their apical plumular bud and assumed a herbaceous habit and bore stems with paired leaves, owing to their selection of new habitats where the competition of other plants demanded the development of a stem with serial leaves so that they could obtain due access to the light. Had they remained prostrate with the single leaf lying on the surface of the ground it is easy to see how they could be overgrown and suppressed by surrounding vegetation.

Specimens of *C. lavandulacea* raised from seed received from the Berlin Botanic Gardens last year, under the name *Roettlera Horsfieldii*, have proved of particular interest in connexion with these studies (Pl. III, Fig. 3). It was assumed they belonged to a temperate region and they were sown in the Alpine Department, the young plants being kept in a frame just sufficiently warmed to exclude actual frost. Only one cotyledon developed, as was to be expected, and this, as in plants grown under 'stove' conditions, was borne eventually on a petiole, some 2.5–3 cm. long; no bud, however, developed in the axil of the cotyledon, so the plants are one-sided only in respect of the single, leafy cotyledon, with nothing to balance it on the opposite side of the stem. The plumule developed normally and the plants grown under these cool conditions have borne either only one or at most two pairs of leaves, and one only or both pairs of the plumular leaves were sessile, flowers being borne in their axils and on their midribs (see Pl. III, Fig. 3).

These cool-grown plants are of interest in comparison with those grown in tropical conditions, since the latter develop a strong shoot in the axil of the cotyledon and also several pairs of large leaves on long petioles, 10–15 cm. long, both on the main stem and on the axillary shoot, before the sessile *Streptocarpus*-like floriferous leaves appear when the plants have reached a height of about 65 cm.

From the behaviour of this species grown in the cool, which results in the suppression of vigorous vegetative growth, it is clear that with a little more suppression such a monophyllous type as that exhibited by Dr. Kerr's specimens of *C. capitis* might well be produced, and the relation between both unifoliate and herbaceous species of *Chirita* and *Streptocarpus* is clearly demonstrated.

The assumption or hypothesis that the caulescent species of *Streptocarpus*,

Chirita, Klugia, &c., have developed from ancestors which adopted the monophyllous habit would appear to be a reasonable one were it not bound up with some puzzling problems of geographical distribution. Nearly all the examples under consideration are found in Africa and Asia, including China, India, and Malaya, while Haberlea and Ramondia occur as Tertiary relicts in the Pyrenees and in the Balkan Peninsula. It is, however, somewhat unexpected to find that the genus Klugia with species in Ceylon and in Central and South America so closely resembles the caulescent species of Chirita and Streptocarpus in shedding one of its two cotyledons with the result that the adult plant is one-sided at the base with its unpaired lowest leaf—the enlarged cotyledon.

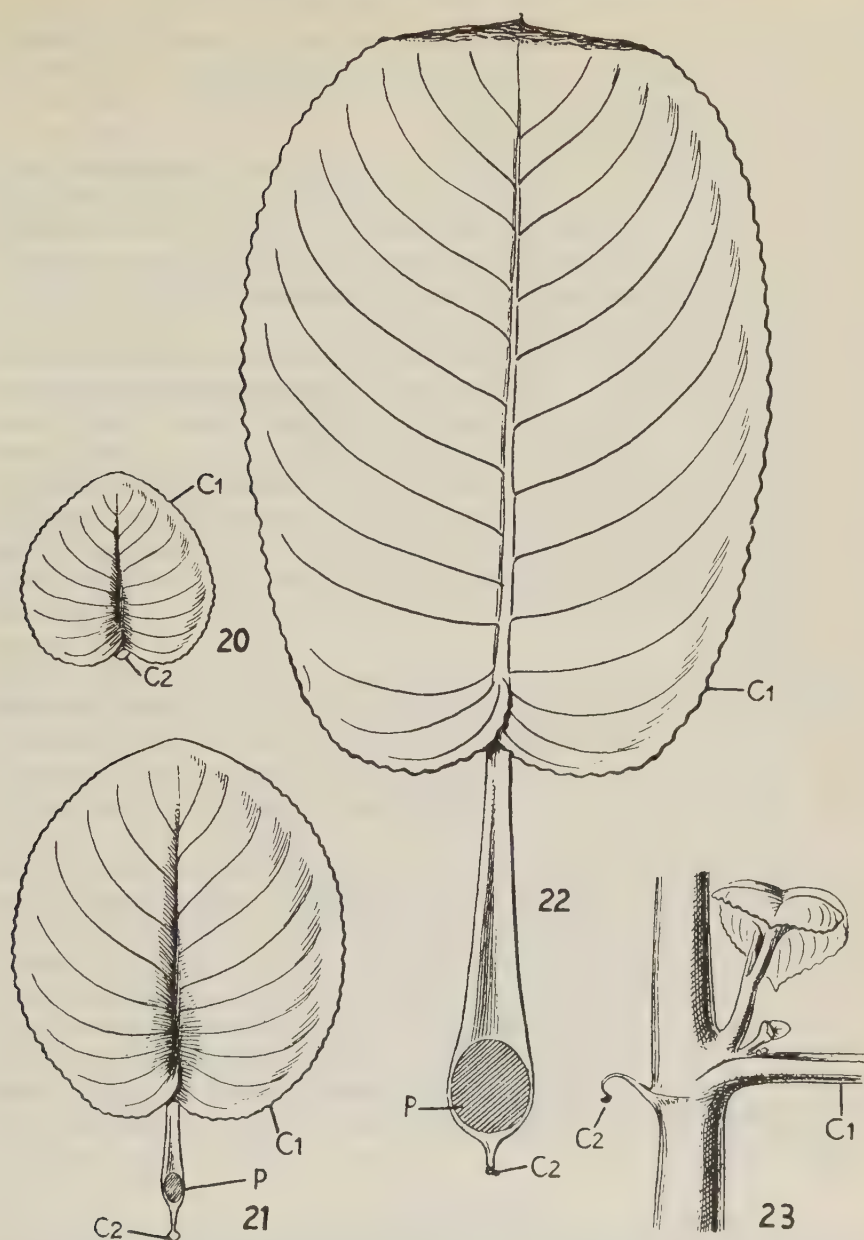
The occurrence of this genus in the New World would render any other explanation of the phenomenon of anisophylly equally, if not more, difficult of acceptance. The fact, however, that in some genera (Haberlea, Ramondia, Saintpaulia, for example) the second cotyledon may sometimes grow to a fair size, though it is always the smaller of the two, and that at times in monophyllous species, such as *Streptocarpus Dunnii*, both cotyledons may occasionally develop, does suggest a partial recovery of a lost habit or function rather than the recent loss of a character—in the case of caulescent species—for which no obvious reason can be suggested.

The cotyledons of Chirita, Streptocarpus, and the other genera whose seedlings have been studied, are, as has been pointed out, at first identical in size, sessile, and glabrous. In the course of a few days the cotyledon which will persist begins to increase in size owing to meristematic activity at its base. The 'adventitious' lamina, which is rapidly developed, soon becomes covered with a fairly dense felt of fine hairs. The actual tip of this leafy organ, however, which represents the actual cotyledon, remains more or less glabrous and distinct, especially in *Streptocarpus Dunnii* and *S. Galpinii*, though it may often become more or less absorbed and obliterated by the basal proliferation which carries the cotyledon at its apex. The second cotyledon which does not increase in size usually remains glabrous, though occasionally a few hairs may be produced on its margin (Text-figs. 1, 2, & 4, and Pl. V, Fig. 11).

Occasionally in *S. Dunnii* and other species, and more or less normally in some species of Didymocarpus and in *Chirita involucrata*, the second cotyledon may persist and develop into a large lamina by meristematic activity at the base as does the other (Text-fig. 5).

In both Streptocarpus and Chirita the cotyledons are sessile or practically so, but in the caulescent species of Chirita and Streptocarpus as the persistent cotyledon develops, not only is there a great increase in the size of the lamina due to the activity of the meristematic tissue at the base of the cotyledon, but a petiole also develops and in some cases attains a length of from 6 to 15 cm. (*C. lavandulacea*), the resultant leaf being exactly similar both as regards its lamina and its petiole to the normal leaves which develop from the plumule (Text-figs. 20–23).

The growth of the 'cotyledonary' petiole continues as the plumular leaves



TEXT-FIGS. 20-23. *Chirita lavandulacea* Stapf. Fig. 20. Seedling in young stage showing the two dissimilar cotyledons. Fig. 21. A more advanced seedling: the enlarged cotyledon (C. 1) has developed a petiole (C. 2, the smaller cotyledon, P. scar of the removed plumule). Fig. 22. A still later stage, the enlarged 'cotyledon' being exactly similar to a plumular leaf. Fig. 23. Part of hypocotyl and plumular stem of a young plant. (All $\times \frac{2}{3}$.) Cf. Pl. III, Figs. 1-4.

develop so that the lamina is carried out into the light and is not unduly shaded by the plumular leaves above it. The meristematic tissue at the base of the persistent cotyledon is thus able to produce both a lamina and a petiole, which exactly resemble a normally produced plumular foliage leaf, the tip only representing the original cotyledon.

That the cotyledon itself does not take part in the great expansion of the lamina can be well seen in some cases where the second cotyledon has persisted and developed. In such cases (see Text-figs. 3 and 4) the actual spatulate cotyledon is seen to be carried at the apex of a somewhat cordate-shaped, pinnately veined, leafy expansion, similar in character to the lamina which has developed from the normally persistent cotyledon.

Repeated experiments have been made with seedlings, both of *Streptocarpus* and *Chirita*, to induce the second cotyledon to develop by constant amputation of the developing persistent cotyledon. This has been attended with some success, especially with *Chirita lavandulacea* (Pl. V, Fig. 13) and more rarely in *Streptocarpus*. In these cases it is of interest to notice that the lamina of the second cotyledon is carried as a small separate glabrous entity at the apex of the broad, hairy lamina which has proliferated at its base. The photograph of the treated seedlings also shows some in which no development of meristematic tissue has taken place at the base of the second dormant cotyledon.

These experiments help to prove the contention that the great development of the persistent cotyledon in these and other monocotylous Gesneriaceae is not due to any growth of the cotyledon itself, but to the activity of the meristematic tissue situated at the junction of the cotyledon and the hypocotyl, the new 'adventitious' growth often engulfing and obliterating the actual cotyledon.

The remarkable development of the 'adventitiously' produced lamina from the meristematic tissue at the base of the cotyledon can be well seen in the single leaf of the genera *Monophyllaea* and *Moultonia*.¹ It is also very marked in certain species of *Streptocarpus* under cultivation. Plants of *S. Dunnii* grown at the John Innes Horticultural Institution, Merton, during the past year developed leaves over 30 in. long by 22 in. wide; those of *S. grandis* had leaves about 27 in. long and 30 in. wide; while the leaves of *S. Wendlandii* measured 17 in. by 20 in. Judging from photographs sent me by Dr. Pole Evans, the single leaves attain very similar proportions on plants growing wild in South Africa.

SUMMARY

The paper opens with a discussion of certain dicotyledonous genera whose seedlings normally possess only a single cotyledon instead of the pair typical for dicotyledons. These include *Cyclamen*, *Ranunculus Ficaria*, *Bunium*,

¹ See Balfour and Smith (1915), where the morphological interpretation of the single leaf of the unifoliate species of *Streptocarpus* has been fully discussed.

Erigenia, *Corydalis cava*, *Pinguicula*, and *Abronia*. In all these cases there appears to be no support for Miss Sargent's theory that the single cotyledon represents two fused cotyledons. Evidence is produced to show that the second cotyledon may be either rudimentary and capable of development (*Cyclamen*) or wholly suppressed (*Ranunculus Ficaria*, *Bunium*, &c.). Reference is also made to the apparently monocotyledonous seedlings of the bulbous *Peperomias*, where one of the two cotyledons never leaves the seed-coat and is a purely absorbent organ.

The various genera of Gesneriaceae, in which one of the two cotyledons of the seedling exhibits continued growth, by means of a basal meristematic zone, are then considered. These include the Asiatic genera *Chirita*, *Moultonia*, *Monophyllaea*, *Didissandra*, and *Didymocarpus* from Indo-Malaya, China and Australia; *Streptocarpus* from Africa, Burma and Siam; *Klugia* from Ceylon, Assam, and America, and several others. Some of these, such as many species of *Streptocarpus* and a few *Chiritas*, *Moultonia*, *Monophyllaea*, *Acanthonema*, &c., possess only one leaf—the enlarged cotyledon—throughout their life-history. Other species of *Streptocarpus* and *Chirita* and also *Saintpaulia* (E. Africa), *Ramondia* (Europe), *Oreocharis* (E. Asia), and others, develop a rosette of leaves but no plumular axis, while the third group including most *Chiritas*, several species of *Streptocarpus*, *Briggsia*, and *Klugia*, and some other genera, consists of herbaceous plants with well-developed leafy shoots. In all these cases one of the two cotyledons ceases to grow at an early stage and aborts and, in the case of the caulescent species, no functional axillary bud is developed in its axil.

It is considered that the unifoliate genera and species represent the primitive conditions and that the caulescent forms are derivatives from ancestors which had assumed the unifoliate habit, and have retained the meristematic zone at the base of one cotyledon when the caulescent habit has been developed.

It is further demonstrated that the single leaf of the unifoliate genera and species described is not really the enlarged cotyledon, since the cotyledon itself can be recognized as a persistent tip in many cases on the large, hairy lamina which is the sole leaf of the plant. This 'leaf' arises as the result of meristematic activity at the base of the petiole, in the region where the persistent cotyledon joins the hypocotyl. As a result of this meristematic activity the large lamina of the *Streptocarpus* leaf, reaching to as much as 30 in. long by 22 in. wide in *S. Dunnii*, or the large 'cotyledonary leaves' with long petioles in *Chirita lavandulacea*, &c., are formed.

In conclusion, I must record my thanks to Dr. Metcalfe for making some anatomical preparations for me in connexion with the adventitious development of the leaf in the monophyllous species of *Streptocarpus*, and for the information he has given me about *Pinguicula* and other matters, to Dr. Cowan, Royal Botanic Gardens, Edinburgh, for the seedlings of *Oreocharis* and *Didymocarpus Mortoni*, to Mr. B. L. Burtt for assistance in working

through the various genera of Gesneriaceae dealt with in this paper, and to Mr. G. Atkinson for taking numerous photographs and for making most of the drawings illustrating this paper, for a few of which I am also indebted to Miss Ross-Craig.

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EXPLANATION OF PLATES III TO V

Illustrating Sir Arthur Hill's paper on 'The Monocotylous Seedlings of Certain Dicotyledons. With Special Reference to the Gesneriaceae'.

PLATE III

Fig. 1. *Chirita lavandulacea* Stapf: a young plant showing the large petioled persistent cotyledon on the left with two axillary shoots and the undeveloped second cotyledon on the right. The plumular leaves are paired and exactly resemble the enlarged cotyledon.

Fig. 2. *C. lavandulacea* Stapf: a flowering specimen. Note the strong development of the shoot in the axil of the cotyledon on the left, also the upper sessile leaves bearing flowers in their axils and along the midrib as in unifoliate *Streptocarpus*.

Fig. 3. *C. lavandulacea* Stapf: grown in a cold frame showing the large cotyledon on the right and only one pair of sessile leaves on the plumular axis bearing flowers.

Fig. 4. A pair of flowering leaves of the plant of *C. lavandulacea* shown in Fig. 1 cut off and seen from above to show the exact resemblance to a flowering unifoliate *Streptocarpus*.

PLATE IV

Fig. 5. *Chirita capitis* Craib growing on an old plastered wall at Bangkok, Siam—only the cotyledon has developed and the plants exactly resemble a unifoliate *Streptocarpus*.

Fig. 6. *C. capitis* Craib growing on limestone rock at Petchaburi, Siam. Here also the plants are unifoliate and the flowers are borne on the petioles and midribs of the cotyledons.

Fig. 7. Plants of the same species grown under good conditions showing the tall branching habit like that of *C. lavandulacea*.

Fig. 8. *Chirita hamosa* R. Br. from E. India, S. China, and Malaya—photographs of herbarium specimens showing examples of unifoliate [(a) and (b)] plants bearing flowers and plants which have developed plumules with pairs of leaves and flowers [(c) and (d)].

PLATE V

Fig. 9. *Chirita involucrata* Craib: seedlings showing the hairs developed on the larger persistent cotyledon and the minute second cotyledon.

Fig. 10. Seedlings of *Streptocarpus Wendlandii* Sprenger for comparison with Text-fig. 1.

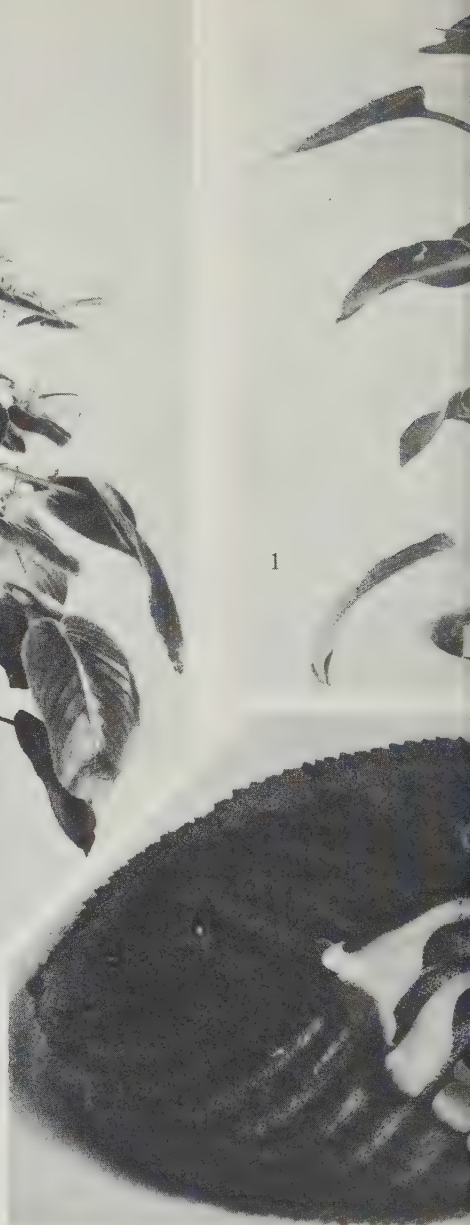
Fig. 11. Seedlings of *Streptocarpus Dunmii* Mast. to show the strap-shaped lamina which develops at the base of the persistent cotyledon with the actual glabrous cotyledon carried as a distinct entity at its apex.

Fig. 12. *Didymocarpus* seedlings showing the enlarging persistent cotyledon carried to a higher level by the development of the mesocotyl.

Fig. 13. *Chirita lavandulacea* Stapf: the persistent cotyledon was cut repeatedly and in a few cases the second cotyledon, which normally dies off, was induced to develop an adventitious lamina at its base, exactly as occurs with the persistent one. The actual cotyledon does not enlarge and is carried as a small glabrous lamina at its apex.



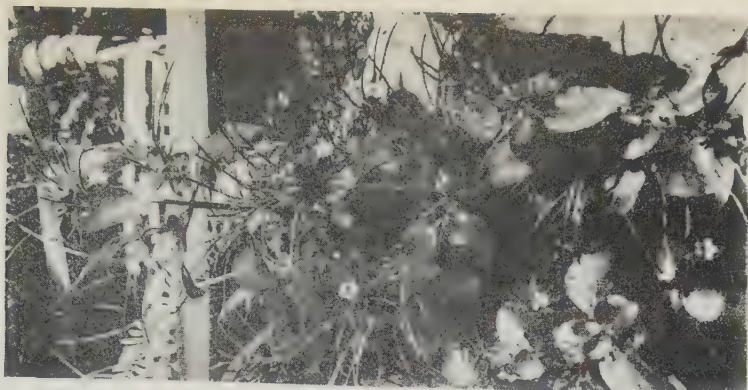
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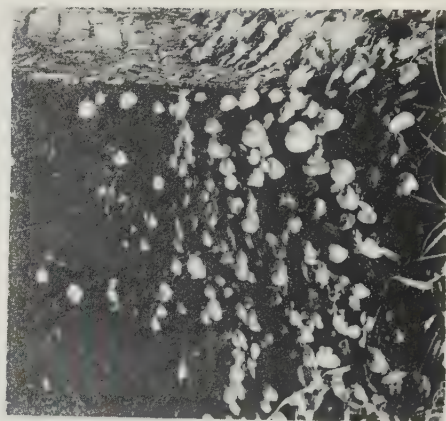
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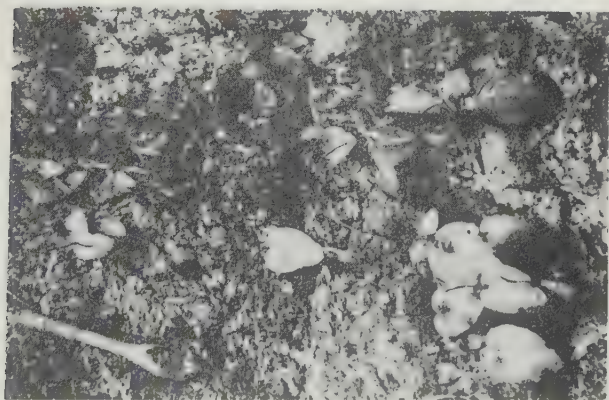
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HILL - SEEDLINGS OF DICOTYLEDONS.



The Morphology and Mode of Development of the Axillary Tubercles and Root Tubers of *Ranunculus Ficaria*

BY

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With twenty-one Figures in the Text

THE tubercles at the cauline and basal leaf axils as well as the root tubers of *Ranunculus Ficaria* are only too familiar to those whose gardens are infested with this plant, because these bodies provide a ready means for the vegetative propagation of the species which is not, therefore, easily eradicated. Hence it is not surprising that this plant has come to be regarded with dislike, and to many its very interesting morphology and the peculiarities in its life history are unknown. Nevertheless, as long ago as 1854 Irmisch studied its morphology and life-history fairly thoroughly. Later on the morphology of the tuberous reproductive bodies was studied by van Tieghem (1866) and Halket (1927). The researches of Marsden-Jones (1935) provided a new stimulus for the study of the species, and the present writer's account of the morphology of the seedling was published in 1936. It is unnecessary to cite more of the papers in which allusion to the tuberous organs of this plant has been made as these are set out more fully in the ones mentioned above. It is interesting to note, however, that Kumazawa (1930) has described the morphology and development of the subterranean tubers of *Ranunculus Zuccarini* Miq., and compared them with those of *R. Ficaria*. He states that in *R. Zuccarini* the first two tubers to be formed on the seedling represent the adventitious roots which arise from the first and second foliar leaves. Additional tubers arise at the bases of succeeding foliar leaves in older plants. The tubers of the Japanese species, unlike those of *R. Ficaria*, are not easily detached from the parent plant. Nor was Kumazawa able to find any tubercles in the axils of the cauline leaves similar to those of *R. Ficaria*, but it is stated in his paper that if the perennial basal part of *R. Zuccarini* is planted rather deeply in the soil, several subterranean internodes are formed. 'In such a case new tubers develop at each subterranean node of the stem, being accompanied by the usual absorbing roots. . . .'

Irmisch's account of the morphology and mode of development of the tuberous bodies in *R. Ficaria* is remarkably accurate considering the poor facilities for microscopical work available in his time. He recognized that both the cauline axillary and subterranean tubers, when mature, consist mainly

of a swollen, starch-containing root, bearing one or more small buds. He states that they arise in all cases as axillary buds in association with which roots are subsequently formed, but the exact mode of origin of the roots was apparently not observed. The buds on the subterranean tubers do not always develop, even after a period of rest, so that these bodies serve mainly as storage organs. In other instances the tubers become separated from one another when the aerial part of the plant dies down during the summer, and then, after a period of rest, give rise to fresh plants by the development of one or more of the buds which have hitherto remained dormant. Fresh tubers are then formed in association with the axils of the leaves of the young plant. Irmisch does not make any distinction between the mode of formation of the subterranean tubers and those in the axils of the cauline leaves, but, as will be shown later on, to have been strictly correct he should have done so. Van Tieghem, likewise, regarded the tubers, on whatever part of the plant they were produced, as adventitious roots which arise in association with axillary buds, and this has been confirmed far more recently by Halket (1927). It is quite common for several tuberous bodies to arise from a single leaf axil on the aerial stem, and when this happens van Tieghem states that each of these bodies is formed in association with a separate bud. Halket (1927) was inclined to agree with this suggestion, but says in her paper that more detailed work would be necessary to confirm this point.

Marsden-Jones has studied the axillary tubercles of *R. Ficaria* from a somewhat different point of view. His paper is not concerned with the morphology of these bodies, but rather with the relationship between their formation and the various degrees of floral reduction exhibited by the plant. He recognized two distinct varieties of *R. Ficaria*, one of which, described as var. *bulbifera*, bears numerous tuberous bodies in the axils of the cauline or basal leaves. The var. *bulbifera* under normal circumstances produces little or no viable seed, although some forms of it are fairly free-flowering, whereas those plants which have no tubercles above soil level produce more flowers and a higher proportion of viable seed. Since Marsden-Jones's paper was published, the causes of the lack of viable seed and the imperfect floral development have been the subject of a more detailed investigation, but this will not be discussed here as it is proposed to publish a separate article on this subject later on.

Marsden-Jones, quoting unpublished work carried out at the John Innes Horticultural Station, refers to the interesting fact that the var. *bulbifera* appears normally to have twice as many somatic chromosomes as the forms without tubercles above soil level. Larter (1932) has also investigated the chromosomes of *R. Ficaria* in material supplied by Marsden-Jones, and also tends to support the same idea. It seems possible, however, that the relationship between chromosome number and the formation of axillary tubercles may not always be quite so simple as it appears to be at first sight. This is suggested by the fact that although Larter found the $2n$ chromosome number generally to be sixteen or thirty-two, and records that the tetraploid plants

alone produce axillary tubercles; he nevertheless says that 'In otherwise diploid roots of *R. Ficaria* isolated groups of tetraploid cells, in which each chromosome type is seen to be represented four times, were found'. In one instance a male plant with sixteen ordinary chromosomes and two fragments was encountered, although most male strains were found to possess perfectly normal chromosome complements.

It is, perhaps, rather unfortunate that the name *bulbifera* has been applied to the variety which bears axillary tubercles above soil level, because the name implies that these are morphologically bulbils or stem structures, whereas they consist in reality of tuberous roots bearing small buds. A more appropriate term for them would appear to be 'tubercle' as employed by Halket (1927), and this name is accordingly adopted throughout the remainder of this paper.

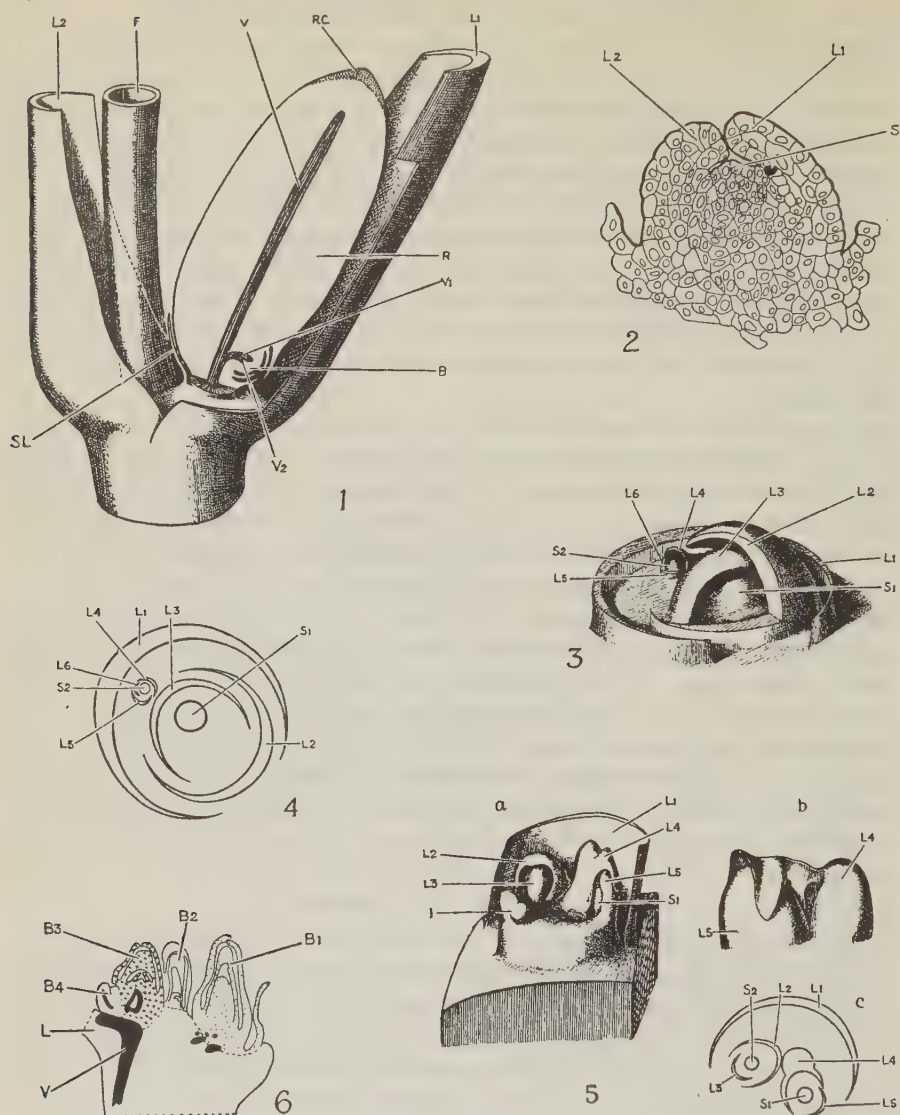
Marsden-Jones's separation of the species into varieties with and without axillary tubercles respectively raises the question of whether there is in fact any fundamental difference in the morphology of the tubercles in the leaf axils above soil level, and the similar-looking subterranean bodies which are common to both varieties. No such distinction was recognized by previous workers, although variations in the degree of floral reduction have been recorded by several workers, some of whom, like van Tieghem, state that the more completely sterile forms bear axillary tubercles whereas the relatively fertile ones do not. At the same time van Tieghem, unlike Marsden-Jones, states that there are tubercles at soil level even in the fertile forms. This uncertainty and the fact that Halket (1927) suggested in her paper that a more complete investigation might yield further information concerning the relationships of the adventitious roots to the axillary buds from which they are believed to arise are the principal reasons why the present investigation was undertaken.

MATERIAL AND METHODS

Most of the material used in this investigation was kindly supplied by Mr. E. M. Marsden-Jones from the Potterne Biological Station near Devizes. Additional wild material from Middlesex, Buckinghamshire, Devonshire, and Dorset was also examined. Fixation was with formalin-acetic-alcohol in which the material was also preserved until required for embedding. Practically the whole investigation was based on serial microtome sections stained in iron-alum haematoxylin and safranin.

Mode of development of the axillary tubercles.

In Fig. 1 a typical example of a simple axillary tubercle is shown, drawn as if it were a transparent body so that the distribution of the vascular strands can be seen. At this relatively mature stage of its development it consists mainly of a large tuberous, negatively geotropic root R, with a well developed root-cap RC. A single vascular strand v, which in cross section shows a typical root structure with alternating xylem and phloem groups, passes into the



FIGS. 1-6. Fig. 1. A fairly mature axillary tubercle drawn as if it were a transparent body so as to show the distribution of the vascular strands. The bases of the adjacent leaves and of a petiole are also indicated. B, main bud of tubercle. F, peduncle. L 1 and L 2, bases of foliage leaves. R, root portion of tubercle. RC, root-cap. SL, scale leaf. V, main vascular strand of tubercle. V 1 and V 2, vascular strands to bud B. Fig. 2. A section of a very young tubercle. L 1 and L 2, embryonic leaves. S, apical growing point. Fig. 3. Diagrammatic reconstruction of a young bud with a secondary one in the axil of one of the component leaves. Fig. 4. Ground plan of buds shown in Fig. 3. Fig. 5a. Diagrammatic reconstruction of a complex system of buds arising in the axil of the leaf L 1. Fig. 5b. Part of 5a viewed from the opposite side. Fig. 5c. Ground plan of Fig. 5a. Fig. 6. A section passing through the base of leaf L in the axil of which there are four buds. Letters in Figs. 3-6. B 1-B 4 buds. L, undifferentiated tubercles. L 1-L 6, foliage leaves. S 1 and S 2, apical growing points. V, vascular strand.

base of the tubercle from the underlying vascular tissue of the parent plant, and extends to the apical growing point of the root. Near the point of attachment to the parent plant there is a small vegetative bud *B*, which is supplied by small branches of the main vascular strand, two of which labelled *v 1* and *v 2* respectively may be seen in the figure. Sometimes a few minute scale leaves, *SL*, are present at the point of attachment of the tubercle to the parent plant. The tubercle is axillant to a leaf, a portion of whose base *L 1* is shown. Fig. 1 also includes the bases of a peduncle *P* and a second foliage leaf *L 2*. During their subsequent development the root portions of tubercles of this kind frequently become positively geotropic, their distal ends growing downwards so that the mature tubercle is frequently curved. Very often the curvaturè brings the root apex in contact with the sheathing base of the leaf in whose axil it has arisen, in which case the leaf base becomes perforated by the pressure exerted on it by the elongating root, which then continues to grow through the hole which is thus formed.

The tubercle in Fig. 1 is a simple type. In other cases it is common to find several tubercles at a single node, or alternatively compound tubercles consisting of a number of buds and roots joined together in a single mass. There seems no good reason for including additional figures of these complex forms as they are well illustrated in some of the previous papers referred to above.

In all cases examined, the tubercles were found to arise from exogenous masses of parenchymatous tissue situated in the leaf axils. These seem to be endowed with an infinite capacity for producing growing points, some of which differentiate as roots whilst others give rise to buds. In a simple case one such axillary mass of parenchyma forms two growing points only, one of which gives rise to a bud and the other a root. Sections through a very young simple tubercle exhibit a structure which does not differ from that of a normal axillary bud, but very soon a root initial is laid down in the tissue of one of the young leaves or in the parenchyma below the stem apex. In some instances the exact point of origin of the root could not be observed, but roots may arise from practically any part of the bud itself or of the tissue immediately beneath it. Later on, the root initial elongates more rapidly than the bud in association with which it has arisen so that the latter becomes progressively less conspicuous during the subsequent development of the tubercle. The bud may eventually be seen as a small, obliquely directed structure at the base of the tubercle as in Fig. 1. A simple tubercle of this kind may, therefore, be regarded as an inverted adventitious plantlet. The very rapid development of the root initial when once it has been laid down makes the earliest stages difficult to follow. The minute scale leaves which are frequently present at the base of a mature tubercle (Fig. 1, *SL*) appear to belong to the original bud but to have become separated from the region of the stem apex by the enlargement of the tuberous root.

The above course of events precedes the formation of a simple tubercle, but, as already stated, more complex forms are very common. One type of

complication results from the formation of additional buds in the leaf axils of the initial bud whilst the tubercle is still attached to the parent plant. It is also common for additional buds to arise adventitiously from the tissues of the tuberous root. By the operation of one or both of these causes it is possible for mature tubercles with one main bud and numerous smaller buds to be produced.

It has already been pointed out that the initial axillary parenchymatous humps, which for convenience may be referred to as protubercles, are endowed with the capacity to produce a considerable number of root and stem initials, so that several tubercles may originate from a single protuberacle. Other complications are due to the fact that some of the initial axillary buds are themselves compound, accessory buds being present in the axils of the leaves of which they are composed. It is quite easy to appreciate from these facts that an infinite variety of forms of mature tubercle are capable of arising according to the arrangement and sequence in which stem and root initials are formed in the original protuberacle. Sometimes a sequence of simple tubercles arises from one axillary mass of tissue, but in other instances the growing points are situated so that fusions between the products of separate stem and root initials are possible. In this way tubercles with extremely complex forms and shapes arise, and when mature it is frequently difficult to unravel the exact manner in which they have developed. Another complicating factor is the enlargement of the tissues contingent upon the accumulation of starch, which sometimes leads to the fusion of originally separate structures.

In one or two instances axillary tubercles were found on which no buds could be detected, in which case the tuberous bodies had the form of simple adventitious roots. This, however, seems to be extremely rare.

Descriptions of specific tubercles of various kinds at different stages of development will now be given to illustrate the above generalizations. All the details in the figures have been observed in serial microtome sections. In the first instance camera lucida drawings of a very large number of individual sections were made in order to illustrate this paper, but it became clear that it was difficult to appreciate their significance unless the actual slides were available for study at the same time. Accordingly rough plasticine models of the buds and tubercles were prepared, with the aid of the serial sections, and drawings of these were made. It is hoped that these somewhat diagrammatic reconstructions taken in conjunction with a limited number of drawings of actual sections will make clear the rather complex nature and mode of origin of the tubercles.

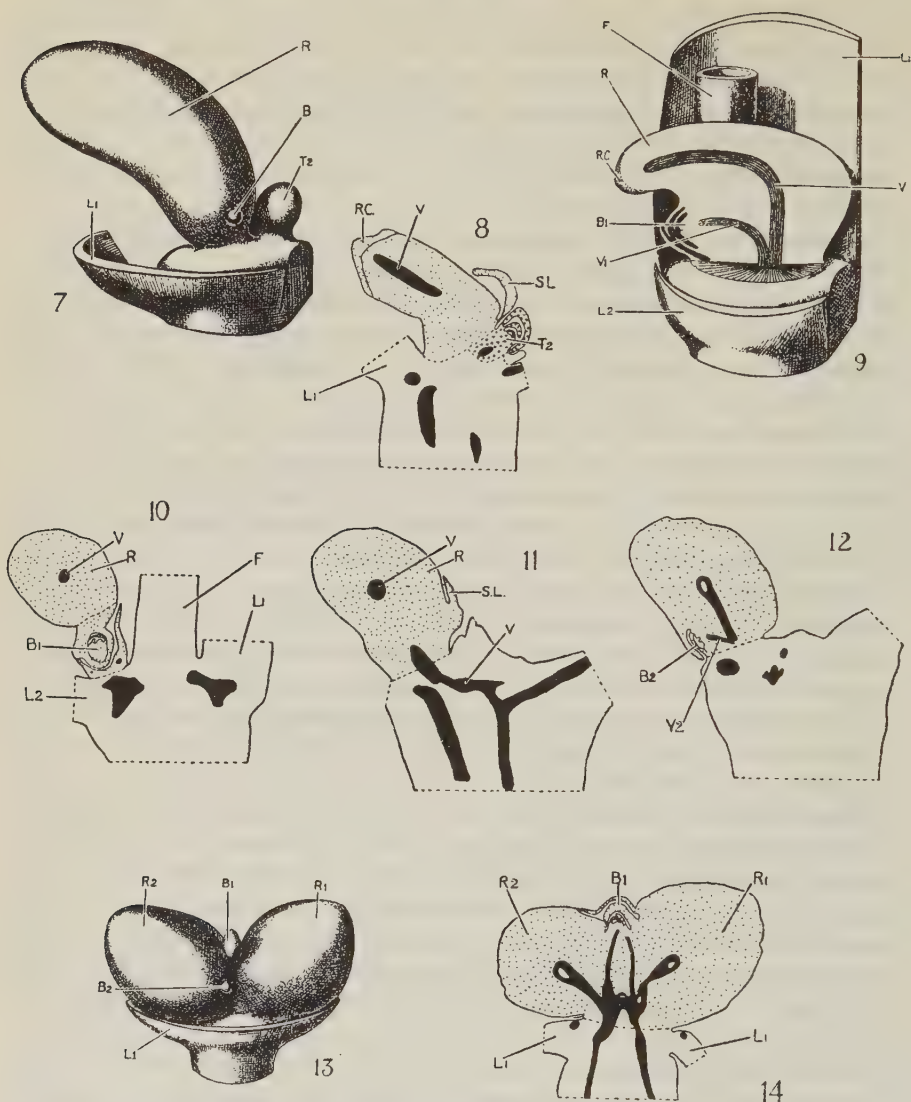
In Fig. 2, which represents a section through a very young tubercle, the stem apex *s* and two embryonic leaves *L 1* and *L 2* are to be seen. In fact the structure is that of a simple axillary bud not completely differentiated, the whole consisting of parenchymatous cells most of which are still filled with cytoplasm and provided with nuclei. In an adjoining section, however, it was possible to distinguish a root initial arising from the bud.

Fig. 3 is a diagrammatic reconstruction of a bud consisting of a stem apex *s* 1 and three leaves *L* 1–*L* 3. In the axil of *L* 1 there is a smaller bud consisting of a stem apex *s* 2 and three leaves labelled *L* 4–*L* 6. Fig. 4 is a ground plan of the same bud system, the same letters applying as for Fig. 3. At this particular stage of development no root initials had been laid down, but it is quite evident that if one or more root initials were subsequently to arise in association with either the main bud or the smaller one a very complex type of tubercle might be formed.

A reconstruction of a slightly older protuberular system is to be seen in Fig. 5. In 5 *a*, *L* 1 represents part of the base of a foliage leaf of the parent plant in whose axil there are two young tubercles arising from a parenchymatous base. In addition there are two rudimentary structures *I*, which exhibited no internal differentiation but probably represented potential tubercles. These might be expected to remain dormant until the older ones had matured and fallen off. Of the older buds shown in the figure, one consists of the embryonic leaves *L* 2 and *L* 3 and the other of the leaves *L* 4 and *L* 5, the centre in each being occupied by the stem apices *s* 1 and *s* 2 shown in 5 *c*, which represents a ground plan of 5 *a*. No root initial was observed in association with the left hand one of the two buds. In the right hand one, however, part of the leaf *L* 4 is almost cylindrical (*L* 4 in Fig. 5 *c*), and its structure indicated that it was a young root arising in association with the leaf *L* 4. In 5 *b* the solid root-like, and the apex of the laminate portions of *L* 4 are also illustrated as they would appear if viewed from the side towards *L* 1. The lamina of *L* 4 has split during the course of its development where it folds over *L* 5 so that there are two flaps of tissue one of which has fallen on either side of the lamina of *L* 5.

Another example in which there are four tubercles *B* 1–*B* 4 arising from a single-leaf axil is illustrated in Fig. 6, which represents one of a series of sections passing through the axillary region of a leaf *L*, which for the sake of simplicity has been depicted as if it were cut off at its base. Part of a very large vascular bundle *v* which supplies two of the young tubercles and then extends upwards into the leaf in whose axil they have arisen is also visible. Very large bundles of this type are always to be found in positions where tubercles are arising.

Having thus seen how the tubercles originate, the various stages of development intermediate between those in 1 and 2–6 respectively will now be considered. When once a root initial has been laid down it develops, as was pointed out above, far more rapidly than the bud and sooner or later begins to curve. Thus in Fig. 7 we have a large curved tuberous root *R* bearing a small vegetative bud *B* which has arisen in the axil of the leaf *L* 1, only the base of which is indicated in the figure. A much younger tubercle *T* 2 is also shown. Fig. 8 is of one of a series of sections passing through a similar pair of tubercles. The root-cap *RC*, vascular strand *v*, and a scale leaf *s.l.* at the base of the larger tubercle are shown. The bud corresponding to *B*



FIGS. 7-14. Fig. 7. A curved tubercle in the axil of the leaf *L* 1 which has been cut off near the base. A younger tubercle, *T* 2, is shown near by. Fig. 8. A section through two tubercles similar to those in Fig. 7. Fig. 9. A view of a curved tubercle drawn as if it were a transparent body shown in relation to the adjacent foliage leaves and a peduncle. Figs. 10-12. Sections of a tubercle similar to that in Fig. 9 taken in three different planes at right angles to that shown in Fig. 9. Fig. 13. A compound tubercle consisting of two roots fused together and bearing two buds, *B* 1 and *B* 2. Fig. 14. A median longitudinal section of the tubercle in Fig. 13. *B*, *B* 1, and *B* 2, buds. *F*, peduncle. *L* 1 and *L* 2, bases of foliage leaves. *R*, *R* 1, and *R* 2, roots. *RC.*, root-cap. *SL.*, scale leaves. *T* 2, young tubercle.

in Fig. 7 was in a different plane from that illustrated in Fig. 8, but the section passes through the bud of the smaller tubercle τ 2, the young root of which could be seen in adjoining sections.

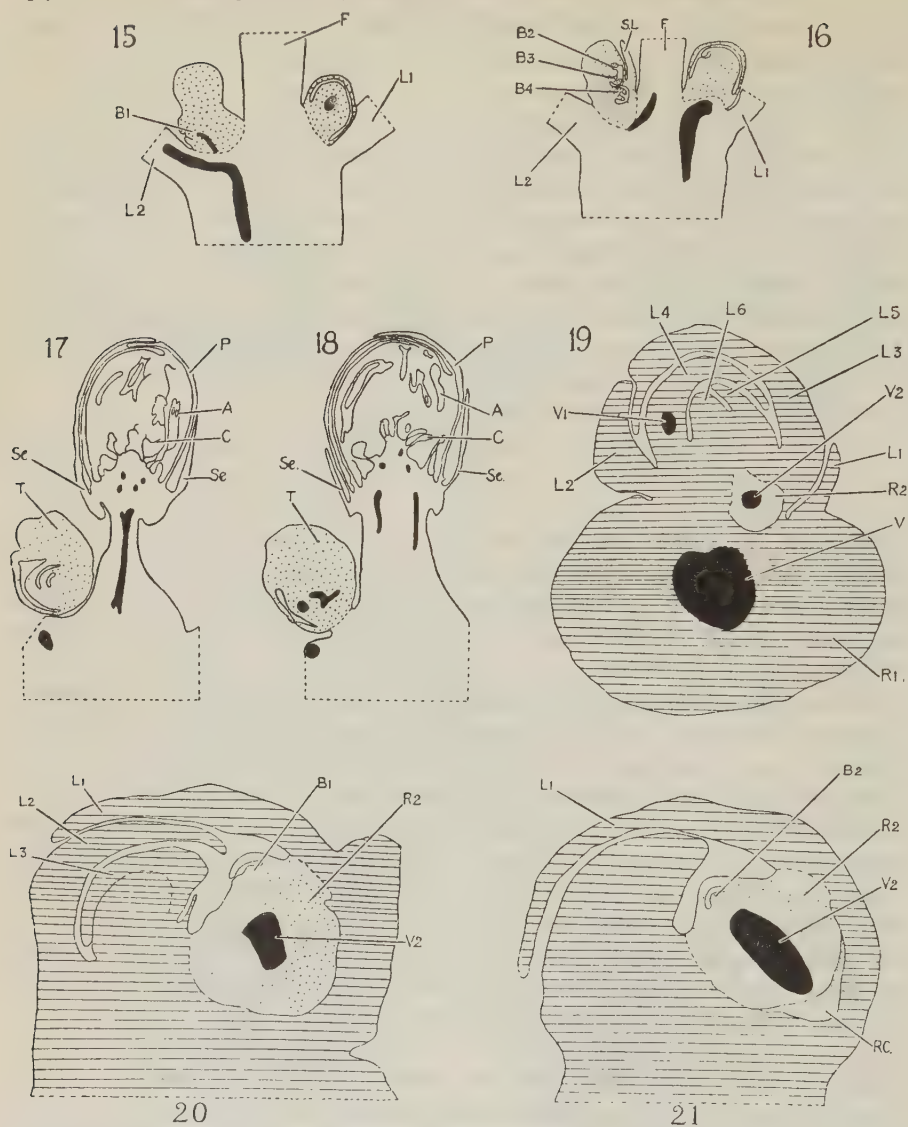
Fig. 9 represents a fairly young tubercle of which the root-tip is orientated in the same direction as the bud B 1, which has arisen in the axil of the leaf L 2, in the region of the peduncle F . The vascular strand v to the root is shown and also one of the smaller strands v 1 passing to the bud. A selection of three sections from amongst a series passing through the tubercle in planes at right angles to the view shown in Fig. 9 are illustrated in Figs. 10–12. A small secondary adventitious bud B 2 which has arisen from the tissues of the tuberous root, and which is supplied by a small vascular strand v 2 is to be seen in Fig. 12. This should not be confused with the main bud B , which is shown in transverse section in Fig. 10.

A tubercle with a single primary bud B 1, a secondary adventitious bud B 2, and two tuberous roots R 1 and R 2 is shown in Fig. 13. A section of the same tubercle is illustrated in Fig. 14. In this instance two root initials must have been laid down in association with the original bud. Figs. 15 and 16 show sections in different planes of two separate tubercles arising in the axils of the leaves L 1 and L 2 on either side of a peduncle F . It is interesting to note that in the left hand one of these, besides the main bud B 1, there are three adventitious buds B 2– B 4 which have arisen from the root tissue.

When axillary tubercles are increasing in size, owing to the deposition of starch in the tissues, the plant frequently appears to be unable to synthesize sufficient food materials to enable the adjacent flowers to develop normally. This may be one of the main causes of the frequent incomplete floral development which is especially prevalent in the forms of *R. Ficaria* which bear numerous tubercles in their leaf axils. In Figs. 17 and 18 longitudinal sections in different planes passing through a young reduced flower close to a tubercle τ are depicted. The sepals se and perianth P are fairly well developed, but the anthers A and carpels C reduced to empty bags. Some of the anthers in these figures appear to be unattached. This is because only small portions of them are included in the sections.

Mode of origin of the subterranean root tubers.

It has already been mentioned that previous workers believed the root tubers to have the same structure and mode of origin as the axillary ones which arise above soil level. This, in a sense, is true, but there are certain differences which must be taken into account. In the seedling, for instance, the first subterranean tuber is an adventitious root which arises endogenously from the tissues immediately below the stem apex near the base of the single cotyledon. It, therefore, resembles an axillary tubercle in that it is an adventitious root which arises in association with a bud, the main difference being that the first tuberous root of the seedling is positively geotropic from the start and the bud in association with which it arises is terminal and not axillary.



FIGS. 15-21. Figs. 15 and 16. Sections passing through different parts of a pair of young tubercles situated in the axils of the leaves L 1 and L 2 on opposite sides of a peduncle F. Figs. 17 and 18. Sections in two different planes passing through an abortive flower, situated near an axillary tubercle T. Fig. 19. A transverse section of a subterranean root tuber passing through a bud situated near the point of attachment to the parent plant. A young tuber initial R 2 can be seen arising from the tissues of the bud. Figs. 20 and 21. Sections in two different planes passing through a young subterranean root tuber (dotted area) arising in the region of the main bud of a parent tuber. Two separate buds, B 1 and B 2, are shown arising from the tissues of the young tuber. A, anthers. B 1-B 4, buds. C, carpels. F, peduncle. L 1-L 6, leaves. P, petals. Se, sepals. SL., scale leaf. R 1, root of parent tuber. R 2, daughter tuber. RC, root-cap. T, axillary tubercle. V, V 1, and V 2, vascular strands. All black areas represent vascular tissue.

In addition this apical bud does not normally become so closely woven into the tissues of the mature tuberous root that it breaks away from the parent plant with the root tuber in the event of the latter becoming detached. When the aerial part of the seedling dies down at the end of the season, however, the buds formed in the axils of the leaves remain associated with the root tuber and undergo further development in the following year. The mode of formation of the second and succeeding subterranean tubers of the seedling has not been followed, but careful observations on the mode of development of young tubers on older plants have been made, both on wild material as well as on small plants grown in water culture so that the developing tubers could be kept under observation during a prolonged period.

New root tubers on young plants were found to arise endogenously from root initials, closely associated with the numerous buds which are always present in the leaf axils at soil level. These roots are positively geotropic from the start and perforate the parenchymatous tissue at the base of the plant, which consists chiefly of the leaf bases which are so close together that they form a small solid rootstock. Having thus reached the exterior of the plant the roots enlarge and become tuberous, starch being deposited in the parenchyma of the cortex at the same time. Adventitious buds arise from the cortical tissue meanwhile, mostly near the point of attachment to the parent plant. The buds frequently arise at a very early stage in the development of the root, so that each root tuber, like the cauline axillary tubercles already described, is almost comparable with an adventitious plantlet. The fate of the root tubers is intimately bound up with that of the parent plant on which they are formed. At the end of the season's growth the aerial parts of the plant die down, leaving the root tubers held together by the small rootstock. At the beginning of the following season's growth some of the buds on the tubers give rise to fresh aerial branches, whilst others remain dormant. The food necessary to initiate this growth is obtained from the tubers. In due course fresh root tubers are formed in association with the young branches, and so the plant perennates and increases in size. Individual tubers or groups of them eventually become separated from one another, and then continue an independent existence, the perennating system of the plant thus serving as a means of effecting vegetative propagation.

Very frequently the subterranean part of an *R. Ficaria* plant consists of a number of short root tubers all joined together in a mass somewhat resembling a bird's nest, in addition to the longer unbranched tubers so far described. Careful examination has shown that these complex tuber masses are produced by the successive formation of root initials in association with the dormant buds of pre-existing tubers.

Figs. 19-21 represent sections of subterranean tubers, in association with the principal buds of which young tubers are to be seen at very early stages of their development. The lower part of Fig. 19 consists of the root R 1, and the single large vascular strand v cut transversely near the point of

attachment to the parent plant. The upper part of the same figure represents a bud, whose leaves L 1–L 6 are shown. R 2 (dotted area) is a very young adventitious root which is arising endogenously near the main bud of the parent tuber, and growing obliquely towards the exterior by perforating the adjacent parenchyma. v 2 is the vascular strand of the young root. Figs. 20 and 21 represent two sections in slightly different planes passing through the main buds of an old tuber in association with which a young tuber can be seen at a slightly later stage of development than that in Fig. 19. In Figs. 20 and 21 the root corresponding to R 1 in Fig. 19 of the parent tuber is not indicated. If it were it would be below the broken line at the base of the figure. In the dotted area R 2, which represents the young tuberous root, two adventitious buds B 1 and B 2 have arisen. The vascular strand v 2 is much larger than that at the stage shown in Fig. 19, and there is a well developed root-cap RC. L 1–L 3 represent some of the leaves which make up the bud of the parent tuber. In Figs. 20 and 21 the young buds of the newly formed tuber are so close to the axil of the leaf L 1 of the bud of the parent tuber that it might at first sight be thought that they are axillant to this leaf. That they are more correctly to be interpreted as adventitious buds arising from the young root R 2 may be inferred from the fact that at an earlier stage of development as in Fig. 19 no buds have been differentiated in the tissues of the young root.

It often happens during the subsequent enlargement of the newly formed roots that fresh root initials arise close to the adventitious buds which have originated in the manner just described. This process may be repeated indefinitely without the successive tubers becoming separated from one another unless there is some form of mechanical interference; and so the complex branched masses of root tubers are produced.

DISCUSSION

It seems reasonable to suppose that *R. Ficaria* has, during the course of its phylogenetic development, gradually lost its capacity for normal sexual reproduction; but that the degree of floral reduction varies considerably in different races. In some districts and countries the floral reduction of *R. Ficaria* is known to be more pronounced than in others. An interesting subject for speculation is why this change has taken place and whether it is universally accompanied by the genetical distinction referred to above. Many people now believe that the initiation of adventitious roots is controlled by the hormone-like action of chemical substances synthesized within the plant itself. Bouillenne and Went (1933) have suggested the term rhizocaline as a suitable one to include all such root-promoting substances. They are more generally referred to as auxins, however, because they possess other growth-regulating properties besides that of stimulating root formation. It therefore seems possible that there is a relationship between the content and distribution of rhizocaline in *R. Ficaria* and the number and situations of the axillary tubercles and subterranean tubers which are produced in different races of this species.

An interesting subject for investigation would be whether the artificial introduction of chemicals known to possess root-promoting properties into an *R. Ficaria* plant which was known under ordinary circumstances to be incapable of producing cauline axillary tubercles, would induce the formation of these bodies. Also whether, in the event of success being obtained in experiments along these lines, the genetical alteration would also be induced. It is hoped that it may in due course be possible to conduct experiments to test this idea.

SUMMARY

The tubercles in the axils of the foliage leaves, as well as the subterranean tubers of *Ranunculus Ficaria*, consist when mature of swollen, starch-containing roots bearing one or more buds. The aerial tubercles, which are frequently compound when mature, originate from simple buds or groups of buds in the tissues of which root initials are laid down. The subterranean tubers likewise originate in the region of axillary buds at the base of the plant, but differ from the aerial ones in being positively geotropic from the start. In both categories of tuber additional buds arise adventitiously from the root tissue. The manner in which large, complex masses of subterranean tubers or compound aerial tubercles arise is described in detail. Marsden-Jones's previous description of forms of *R. Ficaria* with aerial tubercles as a genetically distinct variety under the name *bulbifera* is discussed in connexion with the recent hypothesis that the formation of adventitious roots is governed by the hormone-like action of chemical substances synthesized within the plant itself.

I am indebted to all those who have assisted in various ways with this investigation. My thanks are due especially to Sir Arthur W. Hill, for advice on the preparation of the manuscript, to Mr. E. M. Marsden-Jones for supplying material, and to Dr. W. B. Turrill for many helpful suggestions. The illustrations of the reconstructed buds and tubercles were prepared by Mr. G. Atkinson.

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Studies on the Absorbing Surface of Sugar-cane Root Systems

I. Method of Study with Some Preliminary Results.

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With two Figures in the Text.

INTRODUCTION

MOST of the studies hitherto carried out on the root-system of sugar-cane have been qualitative in nature, and have been concerned mainly with the distribution of roots in the soil at different times in the life-history of the cane and under different environmental conditions. Investigations of this type have yielded very important results, and have found considerable practical application in the selection of parents for breeding purposes, in the selection of canes for dry and wet environments, and in the selection of canes for environments infested with the white grub (*Phytalus smithi*, Arrow).

Various field problems, such as the factors affecting good germination of the cuttings, cultural methods, and fertilizer practices, have also been, at least partially, solved, following the application of the findings from such root investigations. Where quantitative methods have been used they have been based exclusively on either root weights, root length, or root numbers. The distribution of roots in the soil by weight has been extensively investigated, particularly in Hawaii, and, within varieties, close correlations between root weights and crop yields have been established in Mauritius. The information that can be obtained from qualitative methods is limited, and the quantitative methods hitherto used all assume a relationship between the factor measured (root weight, length, or number) and the efficiency of the root system in its major function, viz. absorption. Certain available data created a doubt as to whether such a close relationship as was assumed really existed. The most accurate information on the efficiency of a root system is obviously to be obtained from determinations of the surface area actually concerned in absorption. As far as the author is aware, the only previous attempt to determine the spatial distribution of the absorbing surface of root systems is that of Nutman (1934) working with *Coffea arabica*. It appears from the work of this investigator that in coffee the absorbing surface is confined to those roots which are classed as 'feeder-roots'.

In calculating the absorbing surface of the root system Nutman takes the sum of the surface area of the root-hairs plus the surface area of the feeder roots, excluding that due to the presence of root-hairs, as being equivalent to the absorbing surface. No evidence is provided, (a) that the direct surface of the feeder roots is capable of absorbing, (b) that all the root-hairs function. There is little doubt, that absorption is possible by the epidermal cells not modified as root-hairs in their very young state, but when suberization of the hypodermal layers has taken place, it is obvious that absorption through this channel becomes impossible. Nutman gives no data on the longevity of the root-hairs in coffee or whether they function for as long as they survive on the roots. In general, root-hairs survive for a comparatively short period, a few days or weeks (Lundegardh, 1931), but examples are known where the root-hairs survive for several years, although they do not apparently function for so long a period (Miller, 1931).

M^cDougall (1921) observed that the root-hairs of *Gleditsia tricanthos* persisted for several months, and became thick walled and brown in colour. Whitaker (1923) confirmed the work of M^cDougall and found that the root-hairs in certain species of the Compositae persisted for as long as three years. Weaver (1925) also found that, after ten weeks, wheat roots possessed root-hairs on 99 per cent. of their area. Very little information is available on the duration and functional activity of the root-hairs in sugar-cane.

Artschwager (1925) states that the root-hairs in the variety Black Cheribon persist for a considerable period, but that they do not apparently function for the whole of their life. He provides no experimental evidence in support of this latter statement. The importance of the absorbing surface of root systems is well established, cf. Lundegardh (1931), who states: 'The chief necessity for the root is the development of a very large absorptive surface, and this is attained by repeated branching and by the formation of root-hairs.' The problem of determining the absorbing surface of the sugar-cane root system is complicated by the fact that it is not only the fibrous or 'feeder' roots which can take part in absorption. Every root in the system, at least during its young state, plays an important part in absorption. Moreover, it has been found that many sugar-cane roots several months old still bore a considerable number of root-hairs, not only on the younger parts, but on the older parts in proximity to the stool. In fact, every root in which the cortex had not actually decayed still possessed some root-hairs. Artschwager's statement that root-hairs persist for a considerable period in sugar-cane is thus amply confirmed. In the older sugar-cane roots there is a break-down of cortical tissue, resulting in the presence of large air-chambers, separated from each other by narrow bands of persisting cortical cells. These have been described by Artschwager (1925) and by the author (Evans, 1932). Roots in which this break-down of cortical tissue has occurred to an extreme degree still, however, bear root-hairs (Fig. 1). The author considers that these air-chambers facilitate gaseous interchange within the roots, and that the persisting chain of cortical cells

serve for the conduction of water and mineral substances from the piliferous layer to the endodermis. Wilson (1936) has shown that in *Elodea*, *Potamogeton*, *Stratiotes*, *Carex*, and several other plants root-hairs are formed from special cells, differentiated quite near the root-tip, which are distinguishable

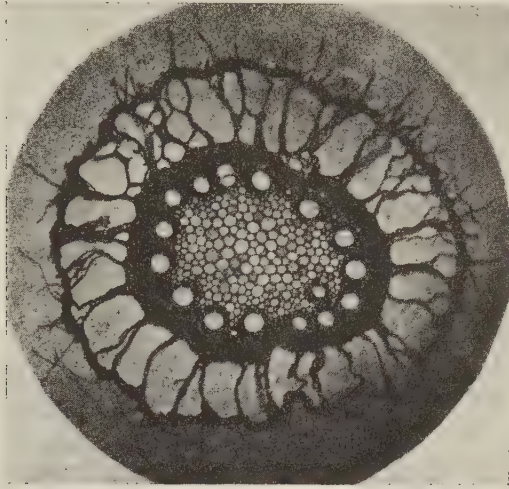


FIG. 1. Transverse section of old root of sugar-cane showing persisting root-hairs.

from the other non-potentially bearing cells of the piliferous layer. Transverse and longitudinal sections of the tip of several sugar-cane roots failed to show a similar specialization of hair-forming cells in this plant.

In order to obtain some indication as to whether the root-hair surface could be taken as an index of the absorbing surface, some preliminary experiments were carried out to determine whether the root-hairs on the older roots were capable of absorbing. Two methods were used, viz. (1) plasmolysis of the root-hairs, (2) absorption of a solution of a dye by intact roots while still attached to the plant.

Plasmolysis of the root-hairs on young, actively growing roots was first effected. It was found that plasmolysis, even in these young hairs, varied from a perfectly regular type to an extremely irregular type. Where the latter was extreme it was often difficult to determine whether plasmolysis had occurred at all. The regular and irregular types of plasmolysis produced are shown in Fig. 2, *a* and *b*. In the first case the protoplasm recedes in one mass from both the lateral and end walls; in the second case the protoplasm becomes broken up into smaller masses.

In the hairs of the older roots plasmolysis was mainly of the irregular type. A few root-hairs on the oldest roots had definitely ceased to function, being filled with air, but the number of these was comparatively small. It is probable that there is a difference in the efficiency of root-hairs for absorption, the efficiency decreasing with age. The evidence of plasmolysis, though not

conclusive, points to the result that even the hairs on the older roots are capable of absorption, if only to a limited degree.

Intact roots of the old type, and of the young actively growing type, were unearthed and placed in a solution of acid fuchsin in separate containers.

After a period of twenty-four hours the young root had absorbed 20 c.c. of the dye solution, whereas the old root had absorbed 4 c.c. The old root was, therefore, capable of some absorption.

It is thus considered that the root-hair surface may be taken as a close approximation to the absorbing surface of the root system. Naturally, in young root systems, where the number of white unsuberized roots is high, the area of the unsuberized surface must be added to the root-hair surface, to obtain an evaluation of the absorbing surface. After a time, however, this direct surface becomes negligible in comparison to the root-hair surface.

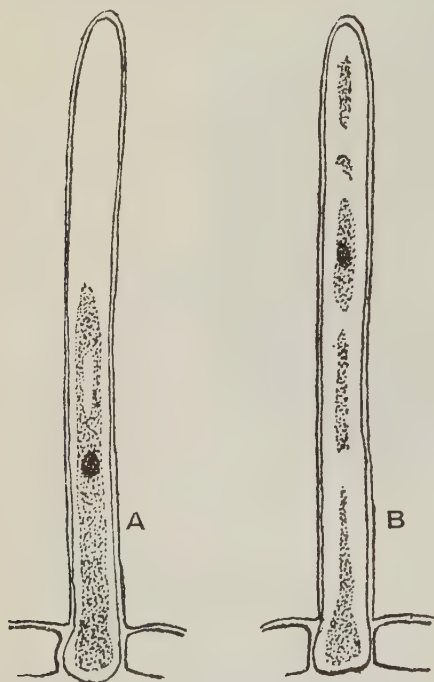


FIG. 2. Types of plasmolysis in the root-hairs of sugar-cane. A = regular; B = irregular.

planted at 9 ft. apart between the stools and 12 ft. between the rows in a root-investigation plot. The qualitative root development had been followed at two, three, five, and six months of age, and the extent and distribution of roots at these stages has been described (Evans, 1936). The results to be described here represent, therefore, further studies of these plants when the root system was more or less mature (twelve to fourteen months old).

MATERIAL

The material investigated consisted of stools of three important commercial varieties: POJ. 2878, BH. 10 (12), and White Tanna. They had been specially

METHOD

Since the technique which was used is original in several respects, the method of determining the absorbing surface will be described in some detail.

A sector of the root system generally consisting of one-third to one-half of the complete system was examined. Taking the centre of the stool as the apex of the sector, arcs were drawn of radius 1 ft., 2 ft., 3 ft., &c., to a maximum distance of 9 ft. in such a direction that there was no interference from neighbouring stools. The soil and roots in each of these areas was carefully removed, first, to a depth of 1 ft., until the whole sector had been completed.

The same procedure was then followed for the remaining foot layers to a depth of 6 ft. Knowing the angle of the sector, the roots in the whole system can then be evaluated. On the soil and roots which had been excavated from these zones, the process of extracting the roots from the soil was then begun.¹ For this purpose a large steel box was constructed (the steel being obtained from empty oil drums of 50 gallons capacity). The box stood on four legs, two legs being 6 in. shorter than the other two legs, thus giving a slope to the box. At the lower side of the box three large circular holes were made, fitted by large rubber stoppers. The dimensions of the box were 6 ft. $\times$ 4 ft. $\times$ 1½ ft. A large quantity of soil can be dealt with in such a box.

On the soil (plus roots), which was placed in the box, a jet of water at considerable pressure was played, breaking up the lumps, and churning up the soil, until the water was about a foot deep in the box. The roots, which were thus freed from the soil, either floated or remained in suspension in the water. The large rubber corks were then removed one by one, the water and roots which escaped passing through a fine mesh sieve. Five such washings to each filling of soil effected a quantitative extraction of the roots from the soil mass, even the most delicate fibrous roots, which could not normally be extracted. On a sample of the fresh roots measurements of the mean length and diameter of the root-hairs were made. From the mass of roots obtained from each zone, after air-drying, the fibrous roots were picked off by hand, the major roots being then graded into three classes according to their diameter. There are thus four classes of roots: group I, fibrous roots; group II, thin, mainly secondary roots; group III, somewhat thick, mainly primary roots or stout secondaries; group IV, thick roots, mainly buttress roots (Evans, 1936). The mean diameters of the roots in the various classes were as follows: group I, 0.20 mm.; group II, 0.5 to 0.7 mm.; group III, 0.8 to 2.5 mm.; group IV, over 2.5 mm. An adequate sample of the various classes of roots from each zone was taken and immediately placed in 70 per cent. alcohol, the air being removed under a suction pump. These samples were kept for subsequent root-hair counts.

The total length of the roots of groups II, III, and IV in each zone was determined by direct measurement. Mixed up with the fibrous roots there was a considerable amount of foreign matter which floated in the water during the extracting process, and which was subsequently retained in the sieve. An efficient method of removing this foreign matter (which consists mainly of lumps of charcoal and other vegetable remains) was devised. The fibrous roots and foreign matter were air-dried and placed in a long glass tube, 1½ metres long and about 1½ in. in diameter. This tube was fitted with a cork and glass tube at the lower end, and a piece of muslin tied around the upper end. A current of air from a blow-pipe was sent in through the glass tube at the lower end. By this means the fine roots were blown higher up the tube

¹ In the preliminary work the roots were picked out by hand, but this method was later replaced by the water-extraction technique described here.

than the foreign matter, which generally was deposited at the bottom of the tube when the air blast was stopped. A complete separation of the roots and foreign matter was thus effected. It was then necessary to determine the total length of the fibrous roots. Direct determination by measurement was impracticable owing to their fineness and the time which would be involved. An indirect method was, therefore, devised for this purpose. The diameter of a large number of fibrous roots was first determined by arranging them serially on a glass slide, and making the measurements under the microscope, using a micrometer scale. The diameter of 200 or more can be determined in a few minutes, thus giving a good estimate of the mean diameter. The fibrous roots were then placed in an accurately graduated flask (the volume of the flask depending upon the amount of roots). Another flask of equal volume was filled to the mark with paraffin oil (kerosene), and sufficient was then poured into the flask containing the roots to bring the volume to the mark. Air-bubbles were removed by revolving the flask gently. The volume of paraffin oil which was left, being equivalent to the volume of the fibrous roots, was either determined directly or by weighing after determining the specific gravity. Paraffin oil was used, since there is no marked swelling or contraction of the roots in this medium in the time necessary to make the determination. Water was quite unsuitable for this purpose, since the roots swell to a considerable degree in this medium. Knowing the volume of the roots and the mean diameter, the length was calculated, assuming them to be cylindrical and having a volume of $\pi r^2 H$.

The method was confirmed by directly determining the length of a large number of fibrous roots, which were then weighed. A factor of length per gramme was determined, and from this the total length was calculated. This value agreed closely with that found by displacement of paraffin oil.

The total length of the different classes of roots in the various zones was thus ascertained.

The next procedure was to determine the density of root-hairs on the various classes of roots. The samples which were retained for this purpose were taken through the usual steps to absolute alcohol and thence to xylol and imbedded in paraffin wax. Sections of standard thickness (1/10 mm.) were cut by means of a hand microtome, three or four sections being taken of each root; about sixty roots of each class were sectioned. The number of root-hairs per section was determined, and mean values calculated for the four classes of roots. As has been stated, root-hair measurements were carried out on fresh roots to avoid the possibility of contraction in the imbedding process. If the system is young, and contains an appreciable number of white roots (the epidermis of which is, presumably, capable of absorbing) the white roots are separated and their length and mean diameter determined.

The mean surface area of a root-hair was calculated from the determinations of length and diameter, and from the number of root-hairs per section, the root-hair surface per unit length for the different classes of roots was

obtained, and eventually, knowing the total length of the different classes of roots, the total root-hair surface of the system arrived at. The direct surface area of white roots may be added to this figure, if it is of any consequence.

Thus the extent of the absorbing surface, which is an accurate measurement of the efficiency of the root system in absorption, was obtained.

RESULTS

The results given in all cases refer to the complete root system and not to the actual sector on which the work was carried out. For convenience, the root-hair counts for the different classes of roots in the three varieties investigated are given in Table I.

It will be seen that there are considerable differences in the density of root-hairs in the three varieties (the figures in Table I refer to the number of root-hairs per 1/10 mm. length). These differences, naturally, will affect greatly the absorbing surface, as will be seen later.

I. *POř. 2878*. In Table II the total length of the different classes of roots for each zone are given (lengths throughout in centimetres).

It will be seen that the vast majority of the fibrous roots occur in the first foot of soil. In the case of the other classes of roots the total length below 1 ft. is greater than that in the first foot. In the first foot of soil only one-eighth to one-ninth of the total length of fibrous roots occur in a circle of 1 ft. radius, taking the centre of the stool as the centre of the circle. The greatest length of fibrous roots occurred between 3 and 4 ft. away from the stool, but fibrous roots are present in considerable numbers up to 6 ft. from the stool.

The actual number of roots which were white and actively growing at the time of examination were estimated to be not more than about 3 to 5 per cent. of the total. The surface area of these would be of small significance compared to the surface area of the root-hairs given in Table III below. If, however, the surface of the fibrous roots was capable of absorption, it would be of appreciable significance compared to the surface area of the root-hairs. The yellowish-brown, suberized appearance of most of the fibrous roots, however, suggests that their surface is not directly concerned in absorption.

The total area of root-hairs on the various classes of roots is given in Table III.

It will be seen that roughly 70 per cent. of the total root-hair surface is distributed in the first foot of soil. Below a depth of 2 ft. most of the root-hairs occur in the region 0-2 ft. from the stool. Of the total root-hair surface borne on the fibrous roots, approximately 88 per cent. is distributed at a distance greater than 1 ft. from the centre of the stool. With regard to the increase in absorbing surface caused by the presence of root-hairs, the increase in this variety is about 3.2 fold in the case of the fibrous roots. This value is lower than that found by Nutman for coffee (8.6 fold), and by Schwarz for corn (6 fold) and peas (12 fold). It must be remembered, however, that the latter

TABLE I
Number of Root-hairs per 1/10 mm. Length of Root

Variety.	Class of root.			Mean length of root-hairs, mm.	Mean diameter of root-hairs, mm.
	Class I.	Class II.	Class III.		
<i>POX. 2878</i>					
1st foot	13 (S.E. 3)	5 (S.E. 1)	18 (S.E. 3)	0.28	0.014
2nd foot	13 (S.E. 3)	5 (S.E. 1)	13 (S.E. 3)	0.28	0.014
3rd foot	16 (S.E. 3)	5 (S.E. 1)	13 (S.E. 3)	0.32	0.014
<i>BH. 10 (12)</i>					
1st foot	4 (S.E. 0.6)	8 (S.E. 2.2)	15 (S.E. 2.4)	0.18	0.011
2nd foot	4 (S.E. 0.8)	5 (S.E. 1.5)	4 (S.E. 0.3)	0.16	0.010
3rd foot	5 (S.E. 0.7)	4 (S.E. 0.6)	5 (S.E. 0.6)	0.19	0.011
<i>White Tanna</i>					
1st foot	1.2 (S.E. 0.4)	1.0 (S.E. 0.3)	4.0 (S.E. 0.6)	0.17	0.012
2nd foot	1.3 (S.E. 0.3)	2.6 (S.E. 0.6)	2.6 (S.E. 0.8)	0.16	0.009
3rd foot	2.4 (S.E. 0.7)	1.5 (S.E. 0.2)	4.7 (S.E. 0.8)	0.17	0.011
			7 (S.E. 2.0)		
			1 (S.E. 0.2)		
			—		

TABLE II
Total Length of Roots (cm.). Var. PO γ . 2878

[illegible]

TABLE III
Total Area of Root-hairs on the Various Classes of Roots. Var. PO γ . 2878

Lateral distance from stool.						Grand total per foot.	Vertical depth and class of roots.
0-1 ft.	1-2 ft.	2-3 ft.	3-4 ft.	4-5 ft.	5-6 ft.		
7160	10068	5365	11250	7475	13673	—	Cl. I } Cl. II } 0-1 ft. Cl. III } Cl. IV }
1063	800	538	1203	1190	1585	—	
4775	1633	1155	1713	1438	2930	—	
1925	830	568	575	1665	1445	89236	
813	1018	1388	640	753	3165	—	Cl. I } Cl. II } 1-2 ft. Cl. III } Cl. IV }
345	480	540	623	520	1318	—	
958	1030	1033	1685	655	2655	—	
1118	118	158	138	210	688	24431	
543	328	95	143	108	278	—	Cl. I } Cl. II } 2-3 ft. Cl. III } Cl. IV }
133	165	45	83	45	168	—	
565	83	70	60	120	360	—	
nil	60	nil	nil	15	158	4101	
85	920	nil	nil	nil	610	—	Cl. I } Cl. II } 3-4 ft. Cl. III } Cl. IV }
113	150	45	18	30	107	—	
248	198	43	25	nil	40	—	
nil	28	nil	nil	nil	nil	2746	
588	814	nil	nil	nil	nil	—	Cl. I } Cl. II } 4-5 ft. Cl. III } Cl. IV }
72	146	13	10	23	45	—	
174	436	34	18	25	90	—	
8	34	nil	nil	nil	nil	2570	
312	295	nil	nil	nil	nil	—	Cl. I } Cl. II } 5-6 ft. Cl. III } Cl. IV }
49	101	14	6	13	32	—	
170	189	31	14	28	119	—	
nil	nil	nil	nil	nil	nil	1427	
Grand total area of root-hair surface 124511 sq. cm.							

TABLE IV
Concentration of Root-hair Surface per Unit Volume of Soil. Var. POJ. 2878

Lateral distance from stool.						Class of roots and depth.
0-1 ft.	1-2 ft.	2-3 ft.	3-4 ft.	4-5 ft.	5-6 ft.	
Beyond 6 ft.						
7160	3356	1073	1607	831	400	304
1063	267	107	172	132	110	35
4775	544	231	245	160	83	65
1925	277	114	82	185	63	32
Total (14923)	(4444)	(1525)	(2106)	(1308)	(656)	(436)
813	339	278	91	84	125	70
345	160	108	88	58	73	29
958	243	207	241	73	145	59
118	39	32	20	23	21	13
Total (2234)	(881)	(625)	(440)	(238)	(364)	(171)
543	109	19	20	12	10	6
133	55	9	12	5	4	4
565	28	14	9	13	24	8
nil	20	nil	nil	2	5	4
Total (1241)	(212)	(42)	(41)	(32)	(43)	(22)
85	307	nil	nil	nil	nil	14
113	50	9	3	3	4	2
248	66	9	4	nil	4	1
nil	9	nil	nil	nil	nil	nil
Total (446)	(432)	(18)	(7)	(3)	(8)	(17)
588	271	nil	nil	nil	nil	nil
72	45	3	1	3	2	1
174	145	7	3	3	2	2
8	11	nil	nil	nil	nil	nil
Total (842)	(472)	(10)	(4)	(6)	(4)	(3)
312	98	nil	nil	nil	nil	nil
49	33	3	1	1	1	1
170	63	6	2	3	4	3
nil	nil	nil	nil	nil	nil	nil
Total (531)	(194)	(9)	(3)	(4)	(5)	(4)

worker obtained his figures on roots grown in moist air. Sugar-cane roots have an increase of 8–10 fold when grown in moist air; and very young white roots in the field, under favourable conditions, an increase of 6–8 fold, but the mean for all the fibrous roots, old and young, is only 3.2 fold. The actual concentrations of root-hair surface on each of the different classes of roots per unit volume of soil are given in Table IV (expressed in terms of the volume of soil in the first circle of 1 ft. radius to 1 ft. of depth, i.e. sq. cm. of root-hair surface per π cubic feet).

As might be expected, the concentration of root-hair surface is greatest in close proximity to the stool, since, in passing outwards, the areas of the zones increase in the progression 1, 3, 5, 7, 9, 11, and 45 (this latter figure is obtained since the zone denoted as beyond 6 ft. was actually 6 to 9 ft.).

Some indications of the degree of branching of the roots may be obtained from the figures representing the fibrous roots per cm. length of the roots included in classes II to IV.

*Length of Fibrous Roots per cm. Length of Roots in
Classes II to IV Combined*

Depths: 0–1 ft.	1–2 ft.	2–3 ft.	3–4 ft.	4–5 ft.	5–6 ft.	(Mean all depths)
Lengths: 1.892	0.410	0.452	0.578	0.770	0.513	1.226

These figures confirm in a quantitative manner the findings of qualitative work with this variety, in which the greater degree of branching of roots in the surface soil, as compared to those lower down, has been noted.

II. BH. 10 (12). The stool of BH. 10 (12) examined compared very favourably with that of POJ. 2878 both as regards number and length of canes. In Table V the length of roots of the different classes for each zone are given.

The most striking feature in these results is the much greater length of all the classes of roots in BH. 10 (12) as compared to POJ. 2878. The total length of roots of all types is 17.3 kilometres in BH. 10 (12) as compared to 8.1 kilometres in POJ. 2878; while the difference in total length of fibrous roots (9.64 kilometres for BH. 10 (12), and 4.47 kilometres for POJ. 2878) is of a similar order. In BH. 10 (12), as in POJ. 2878, the greater portion of fibrous roots (roughly 75 per cent.) is located in the first foot of soil. In the first foot of soil only one-eighth of the total length of fibrous roots occurs within 1 ft. of the stool, this proportion being closely similar to that found in POJ. 2878.

The root system of BH. 10 (12) under these conditions of growth (with no competition from adjacent stools) shows a marked superficial spread. Nevertheless, in the deeper layers the system is situated in closer proximity to the stool than that of POJ. 2878, as is shown by a comparison of Tables II and V. The greatest length of fibrous roots also occurred between 2 and 3 ft. away from the stool, whereas in POJ. 2878 most fibrous roots were found in the zone 3 to 4 ft. from the stool.

TABLE V
Total Length of Roots (cm.). Var. BH. 10 (12)

Lateral distance from stool.						Total.	Grand total per foot.	Class of roots.
0-1 ft.	1-2 ft.	2-3 ft.	3-4 ft.	4-5 ft.	5-6 ft.	Beyond 6 ft.		
86776	80580	120092	85304	97936	77480	148296	696464	Cl. I
15336	50804	45676	52064	52676	35272	103512	355340	Cl. II
47720	35284	34684	19884	19023	14624	31856	203080	Cl. III
3308	1036	nil	nil	nil	nil	nil	4344	Cl. IV
53460	24792	19372	17820	22468	23244	34868	196024	Cl. I
9296	14400	4860	8364	7076	13172	27532	84700	Cl. II
7316	7936	10000	9756	4588	7228	14264	61088	Cl. III
336	504	nil	nil	nil	nil	nil	840	Cl. IV
11620	6200	3876	3100	1240	1396	nil	27432	Cl. I
5052	3348	1540	816	268	540	nil	11564	Cl. II
5336	3256	1112	408	404	272	nil	10788	Cl. III
156	nil	nil	nil	nil	nil	nil	156	Cl. IV
7748	6572	3100	768	nil	nil	nil	18188	Cl. I
3788	2452	1924	580	nil	nil	nil	8744	Cl. II
4508	884	464	272	nil	nil	nil	6128	Cl. III
nil	nil	nil	nil	nil	nil	nil	nil	Cl. IV
10072	3840	776	nil	nil	nil	nil	14688	Cl. I
4988	1280	400	nil	nil	nil	nil	6668	Cl. II
3664	876	656	nil	nil	nil	nil	5196	Cl. III
nil	nil	nil	nil	nil	nil	nil	nil	Cl. IV
5424	2712	3100	nil	nil	nil	nil	11236	Cl. I
2340	1700	592	nil	nil	nil	nil	4632	Cl. II
4856	1024	216	nil	nil	nil	nil	6096	Cl. III
nil	nil	nil	nil	nil	nil	nil	21964	Cl. IV

Grand total length of all classes of roots, 1733376 cm.

In appearance the roots of BH. 10 (12) were older and darker in colour than those of POJ. 2878; this feature had also been noted in a previous examination of the mature root system of BH. 10 (12) (Evans, 1935). There were very few white roots in the system at the time of examination. The roots were in such an advanced stage of suberization that it is difficult to imagine any possibility of absorption directly through the surface of the roots. It is considered, therefore, that the root-hair surface is a fairly exact indication of the absorbing surface of the system.

As will be seen from Table I, the density of root-hairs in BH. 10 (12) is considerably less than that in POJ. 2878 at the same age. This is particularly true of the fibrous roots, which are those chiefly concerned in absorption. The difference in the density of root-hairs in class IV in the first foot is due to a difference in the nature of these roots in the two varieties. In POJ. 2878 the roots referred to this class contained a considerable number of very thick superficial roots which were absent from the root system of BH. 10 (12), the class IV roots of the latter variety being almost all buttress roots. The dimensions of the root-hairs were also smaller in BH. 10 (12) than in POJ. 2878. There is, therefore, a great reduction in root-hair surface in BH. 10 (12) as compared to POJ. 2878, so that although the former variety has double the total length of roots of the latter variety, the root-hair surface is not quite two-thirds as great. The values of root-hair surface in BH. 10 (12) are given in Table VI.

In this variety also, over 70 per cent. of the root-hair surface is located in the first foot of soil. There is little doubt that the marked difference in the density of root-hairs in BH. 10 (12) and POJ. 2878 is associated with the difference in the development of the root system in the two varieties.

As has been shown, root development in BH. 10 (12) is active in the early stages, and therefore in the absence of new shoot development very few new roots are formed. In POJ. 2878, on the other hand, root growth is very slow in the early stages, the development of the root system being much more gradual. At twelve months of age, or later, there is thus a greater proportion of young roots in the root system of POJ. 2878 than in that of BH. 10 (12), resulting in a higher average density of root-hairs on the various classes of roots. This difference in the development of the root system has been noted not only in root studies at Reduit but in the examinations of root systems on *Phytalus*-infested lands in different parts of Mauritius. Thus the root system of POJ. 2878 has a few new white roots showing at almost all times during the year, whereas after the first flush of growth BH. 10 (12) has very few new white roots for a considerable period. Jensen (1931) has also shown that the development of the root system of POJ. 2878 is much more gradual than that of most other varieties. The concentration of root-hair surface per unit volume of soil is given in Table VII. As in POJ. 2878, the concentration of root-hair surface per unit volume of soil is greatest near the stool, decreasing

TABLE VI
Root-hair Surface (sq. cm.). Var. BH. 10 (12)

TABLE VII
Concentration of Root-hair Surface per Unit Volume of Soil. Var. BH. 10 (12)

Lateral distance from stool.						Class of roots and depth.
0-1 ft.	1-2 ft.	2-3 ft.	3-4 ft.	4-5 ft.	5-6 ft.	
					Beyond 6 ft.	
2159	668	598	303	271	175	Cl. I } Cl. II } 0-1 ft. Cl. III } Cl. IV }
763	843	455	370	291	159	
4452	1097	647	267	195	124	
288	30	nil	nil	nil	nil	
Total (7622)	(2638)	(1700)	(940)	(757)	(458)	(263)
1330	206	96	63	62	53	Cl. I } Cl. II } 1-2 ft. Cl. III } Cl. IV }
403	236	48	59	38	59	
683	246	187	130	48	30	
29	15	nil	nil	nil	nil	
Total (2595)	(703)	(331)	(252)	(148)	(173)	(79)
289	51	19	11	3	3	Cl. I } Cl. II } 2-3 ft. Cl. III } Cl. IV }
251	53	15	6	1	3	
498	101	21	5	4	2	
14	nil	nil	nil	nil	nil	
Total (1052)	(205)	(55)	(22)	(8)	(8)	(nil)
193	55	15	3	nil	nil	Cl. I } Cl. II } 3-4 ft. Cl. III } Cl. IV }
188	41	19	4	nil	nil	
421	27	9	4	nil	nil	
nil	nil	nil	nil	nil	nil	
Total (802)	(123)	(43)	(11)	(nil)	(nil)	(nil)
251	32	4	nil	nil	nil	Cl. I } Cl. II } 4-5 ft. Cl. III } Cl. IV }
248	21	4	nil	nil	nil	
342	27	12	nil	nil	nil	
nil	nil	nil	nil	nil	nil	
Total (841)	(80)	(20)	(nil)	(nil)	(nil)	(nil)
135	22	15	nil	nil	nil	Cl. I } Cl. II } 5-6 ft. Cl. III } Cl. IV }
116	28	6	nil	nil	nil	
453	32	4	nil	nil	nil	
nil	nil	nil	nil	nil	nil	
Total (704)	(82)	(25)	(nil)	(nil)	(nil)	(nil)

outwards and downwards. Data on the length of fibrous roots per cm. length of the roots of classes II to IV are given below:

*Length of Fibrous Roots per cm. Length of Roots in
Classes II to IV Combined*

Depths: 0-1 ft.	1-2 ft.	2-3 ft.	3-4 ft.	4-5 ft.	5-6 ft.	Mean (all depths).
Lengths: 1.237	1.337	1.219	1.222	1.240	1.047	1.253

These data show very interesting differences in the degree of branching to form fibrous roots in the two varieties under consideration. The length of fibrous roots per unit length of the major root types (which is hereafter termed 'the fibrous root ratio') is almost equal in the two varieties when the whole root system is considered. In POJ. 2878 the ratio for the first foot of soil is considerably higher than in BH. 10 (12). There is a marked decrease in the ratio in the deeper soil layers in POJ. 2878, but the ratio remains remarkably constant with depth in BH. 10 (12).

It may be of interest to record here that fungal mycelia are often found in association with sugar-cane roots. In POJ. 2878 the hyphae have been observed penetrating the cortex of the fibrous roots. In BH. 10 (12) hyphae penetrated the cortical tissues adjacent to the large internal air-chambers. In one particular instance spores were observed in these air-chambers. The spores were dark brown to black in colour, the outer wall being prolonged into peculiar wart-like projections. The fungus has not been identified, but the spores bear some resemblance to those of *Dicoccum asperum* (Corda) Lind., isolated by the botanist and mycologist from the potato plant in 1927.¹ The spores were observed in killed and fixed material, so that it was impossible to make cultures. The recording of hyphae and spores within intact roots of sugar-cane is interesting in view of Constantin's belief that sugar-cane is a mycorrhizal plant.

III. *White Tanna*. This variety, like the two varieties described above, had made good growth, the number of canes per stool being about equal in the three varieties. The length of cane, however, was considerably less, this feature being in accordance with the known performance of White Tanna in this locality. The length of roots of the different classes in the various zones are given in Table VIII.

In the early stages of development White Tanna had definitely more roots than either of the other two varieties investigated. By the time the plants were twelve months old, however, White Tanna occupied an intermediate position between POJ. 2878 and BH. 10 (12), the latter having the greatest length of roots. Over 70 per cent. of the roots of White Tanna were in the first foot of soil, and 93.5 per cent. of the roots in the first two feet of soil. The proportion of fibrous roots in the first foot of soil (about 75 per cent.) is about equal in the three varieties. It is only in close proximity to the stool that any roots

¹ Private correspondence with the Plant Pathologist, Department of Agriculture.

TABLE VIII
Total Length of Various Classes of Roots. Var. *White Tanna*

Lateral distance from stool.						Class total.	Grand total per foot.	Class of roots and depth.
0-1 ft.	1-2 ft.	2-3 ft.	3-4 ft.	4-5 ft.	5-6 ft.	Beyond 6 ft.		
66632	60436	113120	130168	116220	85228	54236	—	Cl. I } 1st foot.
20328	28464	35092	38540	42032	30968	16908	—	Cl. II }
66052	35336	24472	21584	19524	11496	11884	—	Cl. III }
2900	768	448	612	nil	nil	nil	103448	Cl. IV }
38740	21696	27892	18596	13948	12396	18596	—	Cl. I } 2nd foot.
7952	10528	10124	6828	2472	1056	3528	—	Cl. II }
9204	12752	18252	11248	6156	5016	12156	—	Cl. III }
380	160	140	nil	nil	nil	nil	270416	Cl. IV }
12396	6200	2324	1548	nil	nil	nil	—	Cl. I } 3rd foot.
3116	2676	516	1000	nil	nil	nil	—	Cl. II }
2220	2100	284	324	nil	nil	nil	—	Cl. III }
nil	240	nil	nil	nil	nil	nil	34944	Cl. IV }
5424	5424	4648	nil	nil	nil	nil	—	C } 4th foot.
1536	1284	848	nil	nil	nil	nil	—	Cl. I }
1976	2140	620	nil	nil	nil	nil	—	Cl. II }
nil	nil	nil	nil	nil	nil	nil	—	Cl. III }
6072	5424	nil	nil	nil	nil	nil	23900	Cl. IV }
1572	868	nil	nil	nil	nil	nil	—	Cl. I } 5th foot.
1864	2068	nil	nil	nil	nil	nil	—	Cl. II }
nil	nil	nil	nil	nil	nil	nil	17868	Cl. III }
3100	1164	nil	nil	nil	nil	nil	—	Cl. IV }
1012	132	nil	nil	nil	nil	nil	—	Cl. I } 6th foot.
1036	692	nil	nil	nil	nil	nil	—	Cl. II }
nil	nil	nil	nil	nil	nil	nil	7136	Cl. III }
								Cl. IV }

Grand total length, all roots, 1387712 cm.

penetrating to depths of 4 ft. or more are found. These were in fact mainly rope systems (Evans, 1935). It is interesting to note that rope systems had not previously been observed in this variety. The maximum length of fibrous roots occurred in the zone 3-4 ft. from the stool, as in POJ. 2878. As was the case in POJ. 2878 and BH. 10 (12), the proportion of fibrous roots within 1 ft. of the centre of the stool is only about one-ninth of those outside this region. There were very few new white roots in the system at the time of examination, all the roots being yellow to dark brown or black in colour. It does not appear, therefore, that any appreciable direct absorption through the root surface (as distinct from root-hairs) is possible.

As in the other varieties, so in White Tanna, it is the roots of group III that expose the greatest surface. It is not considered, however, that the direct surface is of great interest from the point of view of absorption, and attention will, therefore, be directed to the root-hair surface. The suggestion has been put forward above that the much lower density of root-hairs on the roots of BH. 10 (12), as compared to POJ. 2878 at twelve months of age, is associated with the earlier development of the former variety accompanied by a flush of root growth in the early stages, very few new roots being afterwards formed. If this suggestion is correct, the density of root-hairs in White Tanna should be even less than in BH. 10 (12), since the development of the root system is even earlier in White Tanna, the root system at six months of age comparing very favourably with the mature root system. This is, indeed, the case, as can be seen from Table I, where, for convenience, the root-hair counts are tabulated together. The total area of root-hair surface on the various classes of roots are given in Table IX.

The total root-hair surface in White Tanna is only about one-eighth that of POJ. 2878 and one-fifth that of BH. 10 (12), when the varieties are twelve to fourteen months old. The age factor is important, for there is little doubt that in the early stages the root-hair surface of White Tanna was the highest. This result is obviously wholly dependent on the accuracy of the root-hair counts. In addition to the very large samples taken, separate unnamed samples of fibrous roots were given to an independent observer, who obtained similar results. There is, therefore, a very marked difference between the three varieties in the absorbing surface at twelve to fourteen months of age. In fact, the extent of the absorbing surface bears no relation to the number and length of roots at this age. It is possible that in very young root systems, where all the roots present bear their maximum number of root-hairs, the number and length of roots would give a close indication of the absorbing capacity, but owing to differential development this does not hold for older root systems. It is considered that these results are of paramount importance for the future conduct of root studies. In Table X the values of the concentration of root-hair surface per unit volume of soil are given (sq. cm. per π cubic feet).

The concentration of absorbing surface in White Tanna, as in the other

TABLE X
Concentration of Root-hair Surface per Unit Volume of Soil. Var. White Tanna.

Lateral distance from stool.						Class of roots and depth.
0-1 ft.	1-2 ft.	2-3 ft.	3-4 ft.	4-5 ft.	5-6 ft.	
471	142	156	131	91	55	Cl. I } Cl. II } 0-1 ft. Cl. III } Cl. IV }
120	56	41	32	28	16	
1556	277	115	73	51	25	
120	11	4	4	nil	nil	
Total (2367)	(486)	(316)	(240)	(170)	(96)	(17)
277	52	40	19	11	8	Cl. I } Cl. II } 1-2 ft. Cl. III } Cl. IV }
122	53	31	15	4	2	
141	65	56	25	10	7	
2	nil	nil	nil	nil	nil	
Total (542)	(170)	(127)	(59)	(25)	(17)	(8)
175	29	6	3	nil	nil	Cl. I } Cl. II } 2-3 ft. Cl. III } Cl. IV }
28	8	1	1	nil	nil	
61	19	2	1	nil	nil	
nil	nil	nil	nil	nil	nil	
Total (264)	(56)	(9)	(5)	(nil)	(nil)	(nil)
77	26	13	nil	nil	nil	Cl. I } Cl. II } 3-4 ft. Cl. III } Cl. IV }
14	3	1	nil	nil	nil	
55	19	3	nil	nil	nil	
nil	nil	nil	nil	nil	nil	
Total (146)	(48)	(17)	(nil)	(nil)	(nil)	(nil)
86	26	nil	nil	nil	nil	Cl. I } Cl. II } 4-5 ft. Cl. III } Cl. IV }
14	3	nil	nil	nil	nil	
52	19	nil	nil	nil	nil	
nil	nil	nil	nil	nil	nil	
Total (152)	(48)	(nil)	(nil)	(nil)	(nil)	(nil)
44	5	nil	nil	nil	nil	Cl. I } Cl. II } 5-6 ft. Cl. III } Cl. IV }
9	nil	nil	nil	nil	nil	
29	6	nil	nil	nil	nil	
nil	nil	nil	nil	nil	nil	
Total (82)	(11)	(nil)	(nil)	(nil)	(nil)	(nil)

varieties investigated, is highest in close proximity to the stool. The fibrous root ratio of White Tanna at different depths is given below:

*Length of Fibrous Roots per cm. length of Roots in
Classes II to IV combined*

Depths: 0-1 ft.	1-2 ft.	2-3 ft.	3-4 ft.	4-5 ft.	5-6 ft.	(Mean all depths).
Lengths: 1.54	1.29	1.88	1.84	1.80	1.49	1.50

These figures confirm the qualitative observation made in earlier studies that branching is more prolific in White Tanna than in the other two varieties investigated. White Tanna and BH. 10 (12) differ from POJ. 2878 in that there appears to be no decrease in the fibrous root ratio with depth in the former varieties, whereas in the latter there is a marked decrease.

DISCUSSION OF RESULTS

Previous investigations (Evans, 1936) had shown that the three important commercial varieties POJ. 2878, BH. 10 (12), and White Tanna differed markedly in the early growth of their root systems. White Tanna showed precocious root development, whereas in POJ. 2878 root growth in the early stages was remarkably slow, BH. 10 (12) approximating more to White Tanna than to POJ. 2878. In the present investigation great differences in the total absorbing surface have been established, there being no relationship between the absorbing surface and the total length (or weight) of roots at the time of examination. In this connexion it is interesting to note that Wilson (1936) states that Farr points out that an increase in the superficial area of roots does not necessarily carry with it a proportionate increase in the power of absorption. Substances which tend to pass into the root-hair, whether they be water or mineral salts, must all pass across the base of the hair, and ultimately, of course, across the endodermis. Thus, there will be two independent limiting factors, the area of the hair base and the endodermis. Any increase in the absorbing surface can only be of value when these factors are not limiting, i.e. under conditions of relative deficiency of the substances absorbed. Scott and Priestley (1928) have also put forward the theory that absorption of water and of salts are two separate processes. Thus the external factors could be limiting the absorption of salts, but not of water. Measurements of the absorbing surface do, however, convey some impression of the capacity of the plant in this direction. Particularly is this true in the case of sugar-cane in Mauritius.

It is almost a normal occurrence for the cane to be subjected to periods of drought of varying duration, and in those districts where moisture is seldom limiting, the soil is generally very poor and deficient in the more important mineral substances.

Even in several districts where the conditions are otherwise good, the cane is subjected to the depredations of the root-eating larvae of *Phytalus*. For most of these districts it is considered that a large absorbing surface is an attribute of considerable importance in a cane variety. It appears probable that the

resistance of a variety to drought or to *Phytalus* is connected with the extent of the absorbing surface at the time of incidence of drought, or feeding of the larvae, respectively. For any specified set of conditions there must be a limiting value of absorbing surface necessary to keep the cane plant supplied with sufficient water to maintain its existence. Under *Phytalus* conditions it is conceivable that one variety might reach this critical limit when, say, one-half of its total roots had been devoured, whereas another variety might lose three-quarters of its total roots before reaching the limit. The remarkable fact that POJ. 2878, with a total root length of only 54 per cent. of that of White Tanna, has, at this age, an absorbing system over eight times as extensive, is a case in point. Survival would depend on the extent of the absorbing surface which is left unharmed by the *Phytalus*. The results presented here, are, in fact, in complete accordance with the known behaviour of the three varieties under conditions of heavy *Phytalus* infestation.

With regard to the causes of the large differences in the absorbing surface at this age it appears probable that a major factor is a difference in the cycle or periodicity of growth. It is difficult to imagine that similar differences existed at other and earlier stages in the life-history of the plants. It appears from the investigations of root distribution that in very young plants, White Tanna had probably the largest absorbing surface. Root development in White Tanna was early and vigorous, and in the absence of tillering no new roots could be formed. Most of the roots had thus passed their period of maximum efficiency. In POJ. 2878, on the other hand, root development was slow in the early stages, so that at maturity there was a considerably greater proportion of younger roots. The capacity to form late tillers is also undoubtedly an advantage in maintaining an effective absorbing surface. BH. 10 (12) was intermediate between the two other varieties in the development of its absorbing surface.

Other work, however, indicates that there may be a difference in the root-hair density in different varieties, which is unconnected with differences in the periodicity of growth.

It is considered that the absence of any relationship between root weights, or lengths, and the extent of the absorbing surface, at least at certain periods in the life-history of the plants, is a consideration which is of paramount importance in the future conduct of root investigations.

SUMMARY

1. A method of determining the absorbing surface of sugar-cane root systems is described.
2. The results of the determination of the absorbing surface of the root systems of three important commercial varieties, viz. POJ. 2878, BH. 10 (12), and White Tanna, on one soil type are given.
3. It has been established that for these varieties at twelve to fourteen

months old there is no relationship whatsoever between the total length (or weight) of all roots and the extent of the absorbing surface.

4. The importance of the absorbing surface in the selection of canes resistant to drought and to *Phytophthora* infestation is discussed.

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Physiological Studies in Plant Nutrition

VII. The Role of Fructosans in the Carbohydrate Metabolism of the Barley Plant

I. Materials Used and Methods of Sugar Analysis Employed

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I. INTRODUCTION

THE work on the nutritional responses of the barley plant, which has been in progress in this laboratory for a number of years, has now reached a stage when further progress in the study of carbohydrate metabolism requires an extension to estimations of carbohydrates in parts of the plant other than the leaves. In addition the desirability of an attempt to construct a complete carbohydrate balance sheet has become apparent.

With the object of defining more closely the problems involved in constructing such a balance sheet a rough analysis of a large sample of barley leaves was made, with the following result: Percentage of the dry weight soluble in ether 10, in 95 per cent. alcohol 26.5, in cold water 18.0, leaving a balance insoluble in these solvents of 45.5. From determinations of total nitrogen in the extracts it was estimated that 3 per cent. (of the dry weight) of nitrogenous substances occurred in the alcohol and water extracts, and about 15 per cent. in the insoluble fraction, while sucrose, fructose, and glucose accounted for 12 per cent. in the soluble fraction, and mineral constituents for 10 per cent. The residual insoluble material was presumably cellulose and 'hemicelluloses', while the residual soluble material, 20 per cent. of the dry weight, remained undefined. It will include yellow pigments and water-soluble 'hemicelluloses'. Since it seemed improbable that so large a percentage of the dry weight would occur in these forms, a further analysis

of the water-soluble fraction was attempted, and resulted in the isolation of a fructosan accounting for about half the undefined fraction in the sample analysed. The details of this isolation have been described by Archbold and Barter (1935).

The existence of fructosans in the unripe grains and stems of barley plants was recorded many years ago, and they are generally regarded as secondary condensation products rather than as primary assimilates, since the opinion was held that they did not occur in the leaves (Colin and Belval, 1922). The present isolation from leaves, therefore, reopens the question of the significance of these compounds in the carbohydrate metabolism, and the conditions under which they are formed. Attention has therefore been focused at this stage of the inquiry on this aspect of the carbohydrate problem.

The present paper, which is divided into two parts, deals with the determination of sugars, including fructosans, in the leaves, stems, and ears of the barley plant. At the same time bearing in mind the ultimate aim of constructing a carbohydrate balance sheet, routine determinations have been carried out of the fresh weight, dry weight, and water-insoluble fraction of all the samples analysed. In this first survey of the fructosan distribution, the experimental plants were grown in soil and no attempt was therefore made to include the roots in the analyses. Some preliminary analyses of whole plants grown in sand culture, and including the roots have recently been made by Russell (1937), and it is hoped to extend this method of investigation further.

In Part 1 of the present paper the collection of material and the analytical methods used are described, and in Part 2 the seasonal drift of carbohydrate is discussed, together with some observations on the effect of defoliation and of removal of the ears, on the distribution of sugars in the stems. Some results dealing with the effect of nitrogen deficiency on the fructosan content are also presented.

2. COLLECTION OF MATERIAL

The barley used was that employed in previous investigations in this Institute, namely, a pure line var. Plumage Archer. The plants were grown in soil in the open, during the two summers of 1935 and 1936 at Rothamsted Experimental Station. In 1935 the plot received a dressing of nitrate at the time of emergence of the third leaf, which resulted in the plants making prolific vegetative growth, producing the large dark green leaves associated with heavy nitrogen manuring.

The 1935 experiment was designed to study the seasonal changes in the fructosan content of the leaves and stems, but little fructosan appeared in the leaves at any time, and rather unexpectedly small amounts even in the stems. Attention was thus drawn to the possibility of a marked nutritional effect on fructosan formation, in view of the rather large amount of nitrogen added to the plot.

The leaf samples from which fructosans had previously been isolated were grown with additions of K and P only at the Imperial College Field Station at Slough in the summer of 1933. When it became evident that the fructosans were largely absent from the Rothamsted samples of 1935, the opportunity was taken to collect samples of plants from Slough grown under different manurial treatments. A distinct relationship between nitrogen deficiency and fructosan content was found, and in consequence no further nitrogen was added to the Rothamsted plot in 1936. As a result a greatly increased fructosan content was observed in this season. The relationship between nitrogen manuring and fructosans was further confirmed by an experiment with plants grown in sand at Rothamsted in 1937 (see Part 2).

In 1936 the scope of the experiment was extended to include not only the seasonal drift in sugar, but the distribution of sugars in the different internodes of the stem, and the effect of defoliation and ear removal. These latter conditions were chosen as a means of producing either accumulation or starvation of carbohydrate in the stems (and leaves), other than that resulting from variation in the external nutrient supply.

In 1935 the plants were sown at the end of March and collections begun on April 29 when the third leaves were emerging. At this stage the plot was thinned, leaving about one plant every 4 in., so that the plot consisted of 12 rows of 40 plants each. Duplicate samples were collected at intervals of approximately a week, each sample being made up of twenty plants. The samples were selected at random by drawing numbers, all the numbers being drawn before the experiment started, and the appropriate plants were selected by counting along the rows. In 1936 the plants were sown early in April, and after thinning the plot as above, collections of duplicate samples of ten plants were made, at first at weekly intervals, and later every two or three days, until the last leaf had emerged. The remaining plants were then pruned so that only four to six tillers remained, in order to reduce the labour involved in removing the ears. They were then divided into three groups, again by drawing numbers, from one of which the leaves were removed at the junction of the blade and leaf sheath, and from a second the ears were removed by making a small slit in the sheath and withdrawing the ear and peduncle after detaching at the junction with the main stem. The third group served as controls. Collections were continued every two or three days until the end of July.

The pruning resulted in the appearance of new tillers within a few days. The growth of new tillers was very pronounced in the plants from which the ears had been removed and least in the defoliated plants. On removal of these tillers, renewed shooting occurred for about ten days in the defoliated plants, and till the end of the experiment (July 27, 1936) in the other two groups. These new tillers were removed at the time of each collection from all the remaining plants.

The material collected from Slough in 1935 consisted of plants grown in

soil on plots which had received applications of N, P, and K singly and in combination, and also unmanured controls. Each treatment had been replicated in six randomized blocks and the samples were made up of seven plants collected at random from each of the six plots, making forty-two in all. Duplicate samples were taken for each treatment on three occasions. The sand culture experiment consisted of twenty-five pots each containing three plants. These pots received a third of the nitrogen given to the appropriate controls. The manures given, in grammes per pot, were as follows: Controls NaNO_3 9.1, $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ 2.52, K_2SO_4 1.85, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 1.25, $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ 0.37. Nitrogen deficient NaNO_3 3.03, $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$ 11.5, and other salts as in controls. Duplicate samples of nine plants each were gathered by random selection on each of three occasions, the first being taken at the emergence of the ninth leaf.

All the collections were timed to begin at 10 a.m., and immediately after gathering the main stems (in 1936 the main stems and first tillers) were separated from the rest of the plant and after cutting off the roots the whole sample of main stems was weighed. Leaves were then detached at the junction of the blade and sheath, and ears at the junction with the peduncle. In the earlier samples a slit was made in the sheath to facilitate removal of the ear. The fresh weights of leaves, stems, and ears were separately determined, and each part then cut up into lengths of half an inch or less. After thoroughly mixing the cut material, aliquots were weighed out for the determination of dry weight (10 gm.) and sugars (10 to 20 gm.). The dry weight estimations were carried out by drying at 50°C . overnight, the low temperature being used to avoid fructose destruction. Samples for sugar analysis were preserved in alcohol till required. After extraction of the sugars with water (see p. 187) the residual material was dried at 100°C . and weighed, giving a measure of the material insoluble in alcohol and water.

3. ESTIMATION OF SUGARS

A. *Preparation of plant extracts for sugar analysis.*

The inclusion of fructosans in the estimates of soluble sugars necessitates some modification of the accepted technique employing alcoholic extraction. Fructosans are readily soluble in cold water, but insoluble in 80 per cent. alcohol, and are consequently only partially extracted at the concentration (75 to 80 per cent.) generally used for sugar extraction. A cold water extraction which will include all the sugars therefore becomes necessary. In order to avoid following alcohol treatment with cold water extraction the possibility of drying the material, and employing a single water extraction was considered. Apart, however, from the absence of facilities for the immediate transference of fresh material to an adequate drying oven, it was found that if the conditions of drying were sufficiently rapid to avoid hydrolytic changes, there was little or no destruction of the enzymes themselves. Consequently hydrolysis of sugar occurred during the subsequent water extraction of the

dried material, and could only be completely prevented by a preliminary boiling with alcohol. Since treatment with alcohol was therefore essential the fresh material was preserved in alcohol in the usual way. Instead of continuing the alcoholic extraction, however, the alcohol was poured off and the plant material washed free from alcohol by shaking with several additions of water. Sufficient water was then added to cover the material and the whole shaken in a mechanical shaker for four hours. The resulting water extract was transferred to a graduated flask, to which the residue of the alcoholic extract and washings were added after the alcohol had been removed by distillation under reduced pressure. The residual material was then washed by vigorous shakings with small additions of water and decantation of the washings into the flask, until the required volume was nearly attained. The liquid was finally made up to volume and preserved in a stoppered bottle with a little toluene. Some emulsification of the fats, &c., extracted by the alcohol occurs during the transference to water and the solutions obtained were always opaque.

As a rule 10 to 20 gm. of fresh material were used for each sample, and the final volume of the extract was made up to 250 or 500 c.c. If smaller amounts only were available, the final volume was adjusted so that the total sugar concentration was about 0.15 per cent. If the fresh weight available is too small, the difficulties of manipulation are somewhat increased by the limitation of the water available for washing the residual tissue. The completeness of the extraction was tested by drying the residual material, and re-extracting with water after grinding in a Wiley micro mill. Some results of such re-extractions are shown in Table I. Fermentation tests were made on the second extracts, and showed that 0.03 to 0.07 per cent. (of the fresh weight) of the reducing power was due to non-fermentable substances.

The extraction technique was thus adequate for leaves and young stems, but rather large errors appeared possible in the older more woody stems. To overcome this difficulty Russell (1937) has designed a mill for grinding the wet tissue, which is specially adapted for quantitative work, and is now in use in this laboratory.

B. *Methods of analysis used.*

(a) *Reducing sugars.* The determination of reducing power has been carried out by the Harding and Downs (1933) copper reduction method, and separate estimates of fructose made after oxidation of the glucose by hypiodite, as suggested by Kolthoff. The details of the method followed are those described by van der Plank (1936), to whose paper reference should be made.

The application of these methods to barley extracts has led to difficulties not encountered in the mangold leaf extracts used by van der Plank, and serves to emphasize once more the necessity for carefully testing any procedure for sugar estimation on each new plant extract to which it may be applied.

In the determination of sugars in plant extracts by means of their reducing power two sources of error may arise: (1) There may be present reducing substances other than sugar. (2) The reducing power of sugars towards the reagent used may be affected by the presence of plant constituents not themselves reducing agents. The efficiency of clarification with basic lead acetates,

TABLE I

Total Sugar Content of Water Extracts of Leaves and Stems of Barley

1st extract. Fresh material cut by hand and extracted with cold water after preliminary alcohol treatment (see text).

2nd extract. Residue from 1st extract, dried, milled, and re-extracted with cold water.

Results expressed as percentage of the fresh weight

Leaves.	1st extract.	2nd extract.	Non-fermentable reducing power.
Sample 1	1.73	0.09	
" 2	1.38	0.08	
" 3	1.62	0.04	
Stems.			
Sample 1 (young)	1.35	0.02	For all samples. 0.03 to 0.07
" 2 "	2.19	0.04	
" 3	2.65	0.15	
" 4 (old)	7.68	1.10	
" 5 "	8.74	1.25	

and with charcoal, and of the use of yeast in overcoming these errors is discussed in detail in van der Plank's paper. As a result of his investigation the aqueous extracts of barley leaves and stems were clarified with dibasic lead acetate, using disodium hydrogen phosphate to remove excess lead exactly as described by him, and also treated with charcoal, and with baker's yeast. From the changes in free reducing power observed after the several treatments it was concluded that both types of interference with the sugar estimates occurred in the unclarified extracts. Some examples of these estimates of free reducing power are shown in Table II.

The presence of reducing substances other than sugar is shown by the reducing power observed after fermentation (columns 2a, 2b, 2c, Table II). This residual reducing power is considerably diminished but not eliminated by clarification with either lead or charcoal, and is usually much lower in stem extracts than in leaf extracts. There is a tendency for it to increase as the plant ages, and in mature and woody parts of the stem the values rise to about 0.25 per cent., as high as those found in the leaves. The lowering of reducing power by the presence of substances not themselves reducing is evident from a comparison of the values obtained after charcoal clarification with those in the unclarified solutions (columns 3a and 3c, Table II). In the leaves there is a total loss of reducing power of 5 per cent. after treatment (columns 1a and 1c, Table II), but if allowance is made for the change in the non-fermentable reducing power, the reducing power due to sugar is found to increase by 6.3 per cent. In the stems, where 'interference' is

apparently less, there is a small increase (3.0 per cent.) in the total free reducing power, and consequently a slightly larger increase in that due to sugar (about 7 per cent.).

TABLE II

The Copper Reducing Power of Aqueous Extracts of Leaves and Stems of Barley before and after Clarification with Dibasic Lead Acetate and with Charcoal.

All results expressed as percentage of the fresh weight.

Date.	1. Before fermentation.			2. After fermentation.			3. Differences (1-2).		
	Un- cleared.	Cleared		Un- cleared.	Cleared		Un- cleared.	Cleared	
		with lead.	with char- coal.		with lead.	with char- coal.		with lead.	with char- coal.
	<i>a</i>	<i>b</i>	<i>c</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>a</i>	<i>b</i>	<i>c</i>
<i>Leaves</i>									
4/6/35	0.53	0.46	0.49	0.12	0.09	0.04	0.41	0.37	0.45
18/6/35	0.55	0.51	0.55	0.12	0.08	0.07	0.43	0.43	0.48
26/6/35	0.84	0.77	0.82	0.15	0.14	0.09	0.69	0.63	0.73
3/7/35	0.85	0.68	0.80	0.19	0.10	0.12	0.66	0.58	0.68
10/7/35	0.96	0.72	0.94	0.20	0.11	0.08	0.76	0.61	0.86
24/7/35	1.06	0.84	0.98	0.23	0.13	0.16	0.83	0.71	0.82
Mean	0.80	0.66	0.76	0.17	0.11	0.09	0.63	0.55	0.67
% increase or decrease over uncleared value.	—	-17.5	-5.0	—	—	—	—	-12.7	+6.3
<i>Stems</i>									
18/6/35	0.77	0.85	0.79	0.05	0.03	0.03	0.72	0.82	0.76
26/6/35	0.79	0.82	0.83	0.04	0.03	0.02	0.75	0.79	0.81
3/7/35	1.29	1.21	1.33	0.11	0.05	0.02	1.18	1.16	1.31
17/7/35	1.83	1.95	1.89	0.08	0.07	0.04	1.75	1.88	1.85
24/7/35	1.30	1.38	1.31	0.10	0.13	0.05	1.20	1.25	1.26
Mean	1.20	1.24	1.23	0.08	0.06	0.03	1.12	1.18	1.20
% increase or decrease over uncleared value.	—	+3.3	+2.5	—	—	—	—	+5.3	+7.1

It has frequently been noted that if charcoal is added to plant extracts there is a preferential adsorption of substances other than sugar, and consequently a smaller loss of sugar is found than if pure sugar solutions are similarly treated. The amount of charcoal used in these tests (200 mg. to 25 ml. of a solution containing the extract from 4 gm. of fresh weight per 100 ml.) was found by trial to be the minimum amount required to give a water clear extract on filtration after standing, with frequent shaking, for an hour. In some leaf extracts the filtrate was still somewhat coloured after treatment. Losses of reducing sugar are considered to be negligible after this treatment, since in the stem extracts there is no change in the fructose content and it is

known that charcoal application results in losses of fructose rather than glucose. A correction for any small losses of reducing sugar would of course increase the observed difference on clarification.

Clarification with dibasic lead acetate results in a fall in reducing power in the leaf extracts and a slight rise in the stem extracts, and it was found that in the leaf extracts this loss is entirely one of fructose and is similar to that found by van der Plank in the mangold. In the stem extracts the amount of lead which must be added before the point of maximum precipitation is reached is about half that required in the leaves and no loss of fructose occurs. Consequently the small increase in reducing power is not masked by fructose loss and the values obtained by lead and charcoal clarification are in good agreement. That these increases in reducing power as compared with the values obtained in the uncleared solutions are significant has been shown by the application of the 't' test to the differences between the observations. For the leaves ' t ' = 2.75 (5% point 2.57) and for the stems ' t ' = 3.9 (2% point 3.74).

In Table III are shown the separate estimates of glucose carried out under the same conditions as the free reducing sugar estimates discussed above. The values obtained after lead and charcoal clarification are in good agreement in both stems and leaves and in both cases are higher than the estimates in the uncleared solutions; moreover, the increase is greater than the amounts observed for the total free reducing power. In addition, therefore, to the increase in reducing power on clearing there is also a relative increase in glucose as compared with fructose, and a consequent decrease in fructose. In the leaves where the percentage of free reducing sugars is very small, this increase is as much as 57 per cent. of the value found in the uncleared solution, and in the stems about 15 per cent. The reason for this apparent underestimation of free glucose in uncleared solutions is not clear, and in the present work all solutions have been cleared with charcoal to overcome the difficulty. Two possibilities present themselves: first, owing to the large amount of iodine absorbed by leaf constituents other than glucose there may be incomplete oxidation of glucose, although an excess of iodine equal to the amount absorbed was always maintained; second, the production of reducing substances by alkaline hydrolysis, during the oxidation process, which would tend to over-estimate the fructose. With regard to the first possibility it was found that in the leaf extracts the amount of iodine absorbed was seven times as great, and in the stem extracts three times as great as that required for the glucose itself (compared with only $1\frac{1}{2}$ times as great in the mangold (van der Plank, 1936)). There is some evidence that the uptake of iodine by these other substances is not complete in the time recommended for glucose oxidation in their absence. Competition for iodine may therefore result in the estimates of glucose being too low.

Some confirmation of the correctness of the glucose estimates made in the cleared solutions was obtained by making direct estimates of glucose by

hypoiodite. Van der Plank has suggested that since hypoiodite oxidizes many plant substances other than glucose its action may conveniently be used to test the efficacy of any chosen clarification process, agreement between the

TABLE III

Estimates of Glucose in Aqueous Extracts of Leaves and Stems of Barley before and after Clarification

All results expressed as percentage of fresh weight

Date.	1. Including non-fermentable reducing substances.			2. Excluding non-fermentable reducing substances.		
	Uncleared.	Cleared with lead.	Cleared with charcoal.	Uncleared.	Cleared with lead.	Cleared with charcoal.
	<i>a</i>	<i>b</i>	<i>c</i>	<i>a</i>	<i>b</i>	<i>c</i>
<i>Leaves</i>						
4/6/35 . . .	0.25	0.31	0.33	0.13	0.22	0.29
18/6/35 . . .	0.18	0.30	0.31	0.06	0.22	0.24
26/6/35 . . .	0.40	0.50	0.47	0.25	0.36	0.38
3/7/35 . . .	0.41	0.45	0.47	0.22	0.35	0.35
10/7/35 . . .	0.49	0.52	0.49	0.29	0.41	0.41
24/7/35 . . .	0.54	0.55	0.54	0.31	0.42	0.38
Mean . . .	0.38	0.44	0.43	0.21	0.33	0.34
% increase on clearing	—	+13.6	+13.1	—	+57	—
<i>Stems</i>						
18/6/35 . . .	0.45	0.53	0.49	0.40	0.50	0.46
26/6/35 . . .	0.48	0.50	0.47	0.44	0.47	0.45
5/7/35 . . .	0.73	0.77	0.76	0.62	0.72	0.74
17/7/35 . . .	0.83	0.99	0.90	0.75	0.92	0.86
24/7/35 . . .	0.58	0.65	0.58	0.48	0.52	0.53
Mean . . .	0.61	0.69	0.64	0.54	0.62	0.61
% increase on clearing	—	+13.1	+4.9	—	+15	—

The figures in cols. 2 *a*, *b*, *c* are obtained by subtracting the values of Table I cols. 2 *a*, *b*, *c* from the values in cols. 1 *a*, *b*, *c* above, since there is no evidence of 'apparent' fructose in the non-fermentable fraction.

estimates of glucose obtained by means of hypoiodite and by a copper reagent being taken as an indication that interfering substances have been removed. In the mangold extracts examined by him such agreement was obtained after clarification with dibasic lead acetate; in the barley extracts, however, this was not the case. After treatment with lead the hypoiodite estimates were still three times greater in the leaves and one and a half times greater in the stems, than the copper estimates. Clarification was carried out in solutions containing about 0.15 per cent. of total sugar, this being a convenient dilution for the preparation of extracts in routine work. The discrepancy between the results was slightly lessened by clearing in more concentrated solutions (1 per cent.) (see also van der Plank, 1936), but by no means eliminated. Recourse was therefore made to estimates of glucose

as the difference between the apparent glucose values before and after fermentation with baker's yeast. Some results are given in Table IV, where it will be seen that the bulk of the iodine-absorbing substances remain unaffected by the yeast treatment. If these estimates of glucose are compared with those in

TABLE IV

Estimates of Glucose in Aqueous Extracts of Leaves and Stems of Barley before and after Clarification by means of Hypiodite

All results expressed as percentage of fresh weight

Date.	1. Before fermentation.		2. After fermentation.		Differences 1-2.	
	Uncleared.	Cleared lead.	Uncleared.	Cleared lead.	Uncleared.	Cleared lead.
	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>
<i>Leaves</i>						
3/7/35	2.86	1.46	2.44	1.11	0.42	0.35
10/7/35	3.30	1.80	2.90	1.37	0.40	0.43
24/7/35	2.75	1.18	2.42	0.83	0.33	0.35
<i>Stems</i>						
26/6/35	0.95	1.11	0.57	0.73	0.40	0.38
3/7/35	1.01	0.78	0.31	0.11	0.70	0.77
10/7/35	1.38	1.24	0.63	0.52	0.75	0.72
24/7/35	1.24	0.89	0.71	0.57	0.53	0.32

Table III (columns 2a and 2b) it is found that the values are now of the same order and in some cases in close agreement. No very great accuracy can be claimed for the hypiodite estimates, for the following reasons. Glucose can only be determined as a small difference between two large values of 'apparent' glucose, and further small differences in titration make rather large differences in the apparent glucose, since the 'thiosulphate factor' for the hypiodite method is only 1.11 ml. per mg. of glucose as compared with 4.4 ml. for the copper reagent. In addition in making replicate determinations large variations were occasionally encountered possibly due to the incomplete absorption of iodine in the time used. Taking into consideration these facts it is concluded that the agreement between the two estimates of glucose is sufficiently good to accept estimates by the copper reagent after clarification as reasonably correct, when allowance is made for the non-fermentable fraction. It would also appear that little or no interfering reaction occurs between yeast and the extracts, since it is unlikely that such a reaction would affect the two estimates of glucose equally.

For routine work charcoal clarification appears to offer the simplest method of preparing the barley extracts for reducing sugar determinations. It must, however, be emphasized that this method cannot at present be recommended for general use, and careful trials are necessary before it can be used with confidence. In the present instance the substances interfering with the estimations are quickly and easily removed by charcoal without loss of reducing sugar, and the correctness of the estimates can be checked by comparison

with the values obtained by lead clarification and by fermentation tests. An average value for the non-fermentable reducing power is satisfactory for most work, since the amount is small after charcoal treatment and not subject to much change. The additional labour of large numbers of fermentations can thus usually to be avoided.

(b) *Total sugars.* Total sugars have been estimated after acid hydrolysis and include any fructosan present. Hydrolysis was effected by heating in a boiling-water bath for ten minutes with $N/5$ H_2SO_4 , conditions which are known to complete the hydrolysis of fructosan and avoid destruction of fructose (see Archbold and Barter, 1935).

Charcoal clarification is unsuitable when total sugars are to be determined, since it is impossible to avoid adsorption of sucrose and probably also of fructosan. Moreover, comparison of results obtained before and after lead clarification show that satisfactory estimates of total sugar can be made in the unclarified extracts. The effect of lead clarification is similar to that already noted in the case of reducing sugars, namely, a loss of total sugar but a rise in total glucose in the leaf extracts, and a rise in total sugar as well as in total glucose in the stem extracts. The rise in total glucose in the leaf extracts has the same absolute value as for the free glucose (0.11 gm. of which 0.07 is due to underestimation relative to fructose), while the increase in glucose on hydrolysis is the same in the uncleared and cleared extracts. The decrease in fructose is a little higher than that in free fructose owing to partial removal of fructosan. It is concluded, therefore, that the underestimation of total sugar in uncleared extracts is of the same magnitude as that of reducing sugar; as a percentage of the total this amount is only 2 per cent. and can be neglected.

In the stem extracts there is no change in the total fructose values on clearing, and consequently no removal of fructosan, and the increases of both fructose and glucose after hydrolysis are the same in the uncleared and cleared extracts. The underestimation of total sugar in the uncleared extracts, which falls entirely on the glucose fraction, amounts to about 8 per cent. of the total sugar. The estimates of total sugar, and of the increases in glucose and fructose on hydrolysis of the same leaf extracts as were used for the reducing sugar determinations of Tables II, III, and IV are given in Table V. The differences in the increases before and after clarification have been tested for significance by the '*t*' test, and the fall in the fructose value in the leaf extracts after clearing is alone significant. The fall in total sugar in the leaf extracts, due to fructose loss, and the rise in total sugar in the stem extracts due to removal of interfering substances, are also significant.

In the present investigation the increase in reducing sugars on hydrolysis has been determined in the uncleared extracts, and by adding to this the estimates of free reducing sugars obtained after charcoal clarification, the value for total sugar is derived. Due allowance has been made for the non-fermentable reducing substances. In some experiments the determination

of reducing sugars in the uncleared extracts was omitted in order to lessen the labour of routine estimations. This omission leads to errors up to 10 per cent. in the estimates of the increases of glucose and fructose on hydrolysis, glucose increase being underestimated and fructose increase overestimated. For many purposes this degree of accuracy is sufficient.

TABLE V

Total Sugar Contents of Aqueous Extracts of Leaves and Stems of Barley, and the Increase in Glucose and Fructose on Hydrolysis with N/5 H₂SO₄, before and after Clarification with Dibasic Lead Acetate

All results expressed as percentage of fresh weight

Date.	Total sugar.		Glucose.		Fructose.	
	Uncleared.	Cleared.	Uncleared.	Cleared.	Uncleared.	Cleared.
<i>Leaves</i>						
4/6/35	1.94	1.77	0.68	0.79	0.85	0.61
18/6/35	1.88	1.61	0.51	0.48	0.94	0.70
26/6/35	2.16	2.04	0.78	0.70	0.69	0.71
3/7/35	1.93	1.77	0.68	0.64	0.59	0.55
10/7/35	3.00	2.65	1.08	1.18	1.16	0.86
24/7/35	2.95	2.13	1.02	0.91	1.10	0.93
Mean	2.36	2.00	0.79	0.78	0.89	0.73
<i>Stems</i>						
18/6/35	2.12	2.24	0.40	0.46	1.01	0.96
26/6/35	1.16	1.15	0.11	0.13	0.30	0.23
3/7/35	1.67	1.97	0.17	0.32	0.31	0.47
10/7/35	2.75	2.99	0.38	0.44	0.62	0.67
24/7/35	2.44	2.53	0.44	0.39	0.80	0.89
Mean	2.02	2.18	0.30	0.35	0.61	0.64

The figures for total sugar are obtained by subtracting the values for non-fermentable reducing power in Table II, columns 2a and 2b, from the total reducing power after acid hydrolysis.

In the extracts from early leaves (first, second, and third) where the amounts of free reducing sugar are only of the order of 0.1 per cent. of the fresh weight, satisfactory replication of reducing sugar values could not be obtained in uncleared solutions, and in addition the values for free fructose only were in some cases higher than those for the whole free reducing sugar. There has been no opportunity as yet to examine the possible reasons why hypiodite treatment should remove or diminish the interference with copper reduction in these solutions, and so the reducing sugar values obtained after charcoal clarification have for the present been assumed to be approximately correct. It has, however, frequently been noticed that in the extracts from these early leaves a small reliberation of iodine occurs after the addition of sulphite in the Kolthoff process. Liberation of iodine during thiosulphate titration may therefore be a factor in bringing about the underestimation of reducing sugar in the direct determination with the copper reagent.

(c) *The separate determination of sucrose and fructosan.* It is evident from the foregoing discussion that clarification does not afford a means of separating sucrose and the fructosans, so that estimates of these sugars must depend either on the separate determination of glucose and fructose before and after hydrolysis with acid, or on the selective action of invertase on sucrose. The success of the first method depends on the absence of sources of glucose other than sucrose, for then twice the glucose increase on hydrolysis gives an estimate of sucrose, and the difference between the increases of fructose and glucose an estimate of fructosan. In the second method if sucrose is the only sugar attacked by invertase then sucrose may be determined by the increase in reducing power after invertase action, and fructosan as the difference between the sugar produced by acid and by invertase hydrolysis. In the barley extracts neither of these conditions is fulfilled and thus estimates by either method are subject to errors which vary in magnitude according to the amounts of each sugar present. The reliance to be placed on such estimates is discussed below.

The action of invertase on barley extracts. Using an invertase preparation supplied by the British Drug Houses, complete inversion of sucrose was obtained in one hour using an enzyme concentration of 0.02 per cent., and incubating at 38° C., and the rate of inversion of sucrose was not affected when it was dissolved in a barley extract instead of water. Under the same conditions samples of fructosan isolated from barley were slowly attacked, 5 to 10 per cent. being hydrolysed in an hour.

The susceptibility of the fructosan was further confirmed by the use of more concentrated enzyme solutions (0.1 per cent.) when complete hydrolysis of some samples was obtained in two days. The susceptibility of different samples was found to vary somewhat (Archbold and Barter, 1935), suggesting that fructosans of different molecular structure may be present, although no difference in the chemical composition has been found in samples so far isolated. Estimates of sucrose from the increase of reducing power after invertase action will therefore be too high, the errors being small when little fructosan is present and quite large when much is present. Thus in some leaf samples where the amount of sucrose is relatively high the error was of the order of 5 per cent., while in stem samples containing much fructosan it may be of the order of 30 per cent. The glucose and fructose produced by invertase after an hour in some samples of leaves and stems is given in Table VI, and the figures make it clear that only a very uncertain estimate of sucrose can be obtained in this way.

If extracts containing large amounts of sugar are subjected to the prolonged action of invertase there is a continuous production not only of fructose but of 'glucose' as well, the amounts of 'glucose' being quite large for the first six hours (the term 'glucose' is used to denote all substances which are estimated as glucose after hydrolysis by invertase or acid). Part of this increase in 'glucose' is due to the presence of 6 per cent. of apparent glucose in the

fructosan molecule, but there is usually too large an excess to be wholly accounted for in this way. Moreover, while the fructose finally produced is equal to that obtained by the action of N/5 acid, the 'glucose' falls somewhat short of the value found after acid hydrolysis. It is thus evident that there are

TABLE VI

Increase in Glucose and Fructose in Uncleared Aqueous Extracts of Barley Leaves and Stems by the Action of 0.02 per cent. Invertase for an hour at 38° C.

Results expressed as percentage of fresh weight					
Date.	Increase in glucose. G.	fructose. F.	Excess of fructose over glucose. F—G.	% error on apparent sucrose. F+G.	Approximate fructosan content.
<i>Leaves</i>					
4/6/35	0.68	0.73	0.05	3.5	0.2
26/6/35	0.66	0.62	0.04	3.1	0.2
24/7/35	0.93	0.98	0.05	2.6	0.2
<i>Stems</i>					
18/6/35	0.18	0.38	0.20	35.7	1.0
24/7/35	0.40	0.46	0.06	7.0	0.4
27/7/35	0.92	1.78	0.86	31.7	7.5

sources of 'glucose' other than sucrose in the barley extracts, an observation which is amply confirmed by the fact that the estimates of sucrose as twice the glucose produced by acid (N/5) hydrolysis frequently exceed those obtained as the result of invertase hydrolysis for an hour, a procedure which has already been shown to overestimate sucrose.

The course of hydrolysis by invertase in an extract of barley stems containing 12 per cent. of total sugar is shown in Table VII. In this example the 'glucose' produced by acid (N/5) hydrolysis exceeds that produced in eight days by invertase by 0.32 per cent. (of the fresh weight), while the fructose is only 0.12 per cent. greater than the corresponding enzyme value. When the enzyme hydrolysis was followed by N/5 acid the 'glucose' value was brought up to that found after the action of acid alone. If now the glucose and fructose produced by enzyme action subsequent to the time when sucrose hydrolysis must be complete, be considered, it is found that the 'glucose' is about 15 per cent. of the fructose, a value too high to be accounted for by 'glucose' in the known fructosan. The excess glucose which does not appear to be derived from sucrose or the known fructosan may thus be divided into two fractions, one not susceptible to invertase action, and one which is susceptible but in a much less degree than sucrose.

It has frequently been shown that the increase in reducing power of plant extracts is greater when hydrolysis is effected by acids than when invertase is used, and Phillis and Mason (1933) have pointed out that this difference may be quite large when N acid is used for hydrolysis, and is usually due to

additional 'glucose'. The action of N acid on a stem extract is shown in Table VII, and it is clear from the figures that no additional sugar is produced by the action of the stronger acid as compared with N/5 acid. In the stem extracts, therefore, the estimate of total sugar obtained after hydrolysis with N/5 acid would appear to include all the di- and polysaccharides present. When a leaf extract was subjected to the action of N H₂SO₄ for

TABLE VII

The Progress of Hydrolysis by Invertase and by N/1 H₂SO₄ in an Aqueous Solution of Barley Stems (gathered July 27, 1936) containing a High Percentage of Total Sugar

Results expressed as percentage of fresh weight

Hydrolysis with 0.02% invertase at 38° C.			Hydrolysis with N/1 H ₂ SO ₄ at 100° C.		
Time of reaction, hours.	Increase.		Time of reaction, min.	Increase.	
	Glucose.	Fructose.		Glucose.	Fructose.
½	0.72	1.20	10	2.28	9.88
1	0.92	1.78	20	2.41	9.38
2	1.13	2.57	30	2.41	8.76
4	1.20	3.32	70	2.28	6.76
6	1.42	4.32	120	2.41	3.88
14	1.71	6.89	180	2.41	2.51
24	1.90	6.26			
48	2.03	8.01			
72	1.90	8.76			
96	1.90	9.26			
168	1.99	9.76			
192	2.09	9.76			
Acid (N/5) hydrolysis after 8 days with invertase					
	2.41	9.88			
Acid (N/5) hydrolysis alone					
	2.41	9.88			

3 hours at 100° C. the total glucose present was found to be 2.34 per cent. (of the fresh weight) as compared with 1.46 per cent. after the action of N/5 acid. Furthermore, in leaf extracts containing no fructosan cases have been observed where 'glucose' is produced in excess of fructose even when the weaker acid is used. The differences in these extracts were of the order of 0.15 per cent. In leaves, therefore, there exists yet another source of sugar which can only be determined by hydrolysis with the stronger acid. This fraction has not been included in the present work which deals primarily with the fructosans.

With regard to the 'glucose' produced by invertase and not accounted for as the products of hydrolysis of sucrose and the known fructosan two obvious sources suggest themselves. First, there may be present fructosans containing higher proportions of glucose than the samples isolated by Archbold and Barter (1935). The method used in the purification of these samples led to

the isolation of the least soluble fractions, and the presence of others is by no means excluded. Second, raffinose may be present, and owing to its slower response to invertase action as compared with sucrose, continue to produce apparent glucose after sucrose inversion is complete. Such evidence as there is points to the first of these possibilities as being the most likely. Raffinose is split to fructose and melibiose by yeast invertase, and with baker's yeast the fructose only is removed by fermentation. The reducing value after fermentation, due to the melibiose, will therefore be equivalent to one-third of the amount of raffinose originally present if the calculation is made using a factor for glucose. On the assumption that all the non-fermentable reducing power (0.25 per cent. calculated as glucose) of the stem extract referred to in Table VII is due to residual melibiose the maximum possible amount of raffinose would be 0.75 per cent., of which 0.25 per cent. would be estimated as glucose after invertase action. Under the conditions used for invertase action hydrolysis of raffinose to fructose and melibiose was completed in six hours and in one hour was half completed. The 'glucose' produced in the barley extract between one and six hours was 0.50 per cent., of which 0.19 per cent. could arise from the known fructosan, and a maximum of 0.13 per cent. from raffinose. The amount of 'glucose' actually produced is thus too large to be wholly accounted for as the product of hydrolysis of raffinose, moreover it has already been pointed out that no additional sugar was produced by the action of N acid, conditions which would hydrolyse raffinose to glucose, galactose, and fructose (with some destruction of fructose). In the leaf extract the extra sugar produced by N acid is too great to be accounted for as raffinose, if the estimate of the maximum amount of this sugar which can be present is based on the non-fermentable reducing power. It has therefore been assumed, in the absence of direct proof by isolation, that the additional glucose arising from invertase action is a product of the hydrolysis of a second fructosan containing more than 6 per cent. of 'glucose', although the presence of some raffinose in the leaves is not excluded.

At present the best estimate of fructosan is the difference between the fructose and glucose produced by hydrolysis with N/5 acid, with a correction to allow for the 6 per cent. of glucose known to be present. This estimate will, however, always be low, underestimation arising from the presence of small amounts of apparent glucose from undefined sources, and from fructosans containing a higher content of glucose. The best estimate of sucrose is twice the increase of glucose after invertase action for an hour; this will always be too high a value owing to the production of some glucose from fructosans. Less good estimates of sucrose can be obtained as the total increase after invertase action, or, if only acid hydrolyses are available, as the difference between the total increase on hydrolysis and the fructosan estimate. These estimates will be subject to errors due to hydrolysis of fructosan by invertase and to the glucose content of the fructosans, which may be so large as to invalidate the sucrose values completely.

In many leaf samples the difference between the estimates of sucrose by invertase and as twice the glucose increase on acid (N/5) hydrolysis is quite small, so that only a little glucose arises from sources other than sucrose and the sucrose values are accurate to about 5 per cent. In the stem samples, however, the differences between the estimates increase greatly with age, the estimates from invertase action being lower than those from acid hydrolysis. No valid estimates of sucrose are possible in the later stages. It is evident that this increasing difference indicates an accumulation of the supposed fructosan containing more than 6 per cent. of glucose. This point will be dealt with more fully in Part 2.

In the present work estimates of fructosan are recorded by the method noted above, and sucrose estimates as the difference between the total increase in sugar after hydrolysis with N/5 H_2SO_4 and the fructosan. Where the amount of apparent sucrose determined from invertase hydrolysis was lower than that found by the above method, the enzyme value has, however, been taken as the best estimate of sucrose. In these cases there is a residual value of undefined sugar which serves as a rough measure of the variations in other fructosans or in glucose from unknown sources. The results of acid hydrolysis may be more simply expressed as increases in glucose and fructose irrespective of the source, a method which often serves quite well to demonstrate fructosan changes.

The methods of estimation used may be briefly summarized as follows: free glucose and fructose are determined after clarification with charcoal, by the Harding and Downs copper reagent, coupled with the Kolthoff process for oxidation of glucose. Total glucose and fructose are determined as the increase in these sugars after hydrolysis with N/5 H_2SO_4 plus the values found for the free sugars.

Fructosan is determined as the difference between the glucose and fructose produced on hydrolysis, multiplied by 100/88,¹ to allow as far as possible for the apparent glucose in the fructosan molecule. Sucrose is determined either as the difference between the total increase after acid hydrolysis and the fructosan value, or as the increase of reducing sugar after invertase hydrolysis, whichever gives the lower value.

Clarification is necessary for the estimation of free glucose and fructose, since there is an underestimation of glucose relative to fructose in uncleared solutions. There is also an underestimation of total sugar of about 2 per cent. in the leaf extracts and 8 per cent. in the stem extracts due to interference by non-reducing substances. Allowance has been made for these errors by estimating total sugars as the sum of reducing sugar determined in clear solution, and the increase of sugar after acid hydrolysis determined in uncleared solution. Allowance has also been made for the non-fermentable

¹ This figure is deduced from the equations $G = \frac{1}{2} Ss + \frac{6}{100} Fr$ and $F = \frac{1}{2} Ss + \frac{94}{100} Fr$ whence $F - G = \frac{88}{100} Fr$, where F and G are the increases of fructose and glucose on hydrolysis, Ss is the sucrose, and Fr the fructosan.

reducing power by the use of an average value in the 1936 experiment, and by determinations on each sample in the 1935 experiment.

C. *Sampling errors of the estimates of sugars.*

A series of analyses of solutions prepared from duplicate samples each of ten plants (1936 experiment) are shown in Table VIII. The solutions were

TABLE VIII
Standard Errors of the Estimates of Total and Reducing Sugars in Aqueous Extracts of Barley Leaves and Stems

Solutions prepared from duplicate samples of ten plants each and results expressed as percentage of the fresh weight

Date.	Total sugar.			Reducing sugar.		
	Sample	Difference		Sample	Difference	
	1.	2.	1-2.	1.	2.	1-2.
<i>Leaves</i>						
12/5/36	1.00	0.92	0.08	0.14	0.16	0.02
19/5/36	1.54	1.54	0.00	0.20	0.23	0.03
26/5/36	1.21	1.24	0.03	0.21	0.21	0.00
3/6/36	1.30	1.38	0.08	0.27	0.27	0.00
16/6/36	2.01	1.96	0.05	0.33	0.28	0.05
19/6/36	1.90	2.10	0.20	0.37	0.39	0.02
24/6/36	1.62	1.86	0.24	0.38	0.41	0.03
1/7/36	1.13	1.13	0.00	0.50	0.61	0.11
10/7/36	1.49	1.62	0.13	0.28	0.29	0.01
21/7/36	3.77	3.55	0.22	0.38	0.41	0.03
Standard error of a single difference			0.137			0.045
<i>Stems</i>						
16/6/36	1.33	1.41	0.08	0.63	0.56	0.07
19/6/36	1.16	1.48	0.32	0.53	0.80	0.27
24/6/36	1.89	1.82	0.07	0.75	0.78	0.03
"	1.66	1.32	0.34	0.79	0.76	0.03
"	1.75	1.57	0.18	0.82	0.80	0.02
1/7/36	1.25	1.19	0.06	0.75	0.68	0.07
10/7/36	3.86	3.58	0.28	0.41	0.49	0.09
"	2.38	2.67	0.29	0.81	0.68	0.13
21/7/36	7.68	7.68	0.00	0.32	0.39	0.07
"	8.74	8.79	0.05	0.41	0.37	0.04
Standard error of a single difference			0.219			0.111

prepared as described on p. 186 and total and reducing sugars estimated by the methods discussed above. Replicate analyses of the same solutions agreed to within 1 per cent. From the difference between the results of the duplicate analyses the standard deviation of the difference between any two estimations has been calculated. It will be seen that the errors for the stem samples are rather larger than those of the leaf samples. This may be due to the greater difficulty of completing the extraction of the stems, especially at the later stages of the growth cycle (see p. 188).

4. SUMMARY

1. For the estimation of total sugars, including fructosans, in the barley plant a cold water extract was necessary since the fructosans are insoluble in 80 per cent. alcohol. Using either fresh or dry material, a preliminary treatment with alcohol was essential to avoid hydrolysis by the plant enzymes during water extraction. The technique adopted for the preparation of aqueous extracts is described.

2. Sugar estimations were made using the Harding and Downs micro-copper reagent. Fructose was separately determined after oxidation of glucose by hypiodite. Total sugar was estimated after hydrolysis with N/5 acid, and it is shown that reasonably accurate estimates were obtained in unclarified extracts, but for the estimation of free reducing sugar clarification with charcoal was necessary. Allowance was made for the small amounts of non-fermentable reducing substances present.

3. Unclarified aqueous extracts of barley leaves and stems contain substances not themselves reducing, which not only lower the reducing power of the free reducing sugars by about 5 per cent., but appear to prevent the complete oxidation of glucose by hypiodite. These interfering substances may be removed either by dibasic lead acetate or by charcoal. In barley extracts charcoal clarification can be effected without loss of reducing sugars and offers a quick and efficient method of clarification for routine work.

4. Treatment of the extracts with N/5 acid for ten minutes hydrolyses the fructosans and sucrose, resulting in a production of an excess of fructose over glucose.

5. The prolonged action of invertase yields an amount of fructose equal to that produced by N/5 acid, showing that the fructosans are susceptible to this enzyme. The amount of glucose produced is a little less than that produced by N/5 acid.

6. The known fructosan of barley contains 6 per cent. of 'glucose', but the amount of 'glucose' produced either by N/5 acid or by the prolonged action of invertase is too great to be accounted for solely as the result of hydrolysis of sucrose and this fructosan. The presence of a second fructosan containing more than 6 per cent. of 'glucose' is suggested to account for the excess 'glucose'.

7. Hydrolysis with N acid for three hours yields no additional 'glucose' above that found after the action of N/5 acid in stem extracts, and the presence of raffinose is thus excluded. In the leaf extracts a considerable extra amount of 'glucose' is found after the action of N acid. Fermentation tests showed that this additional glucose could only be due in part to the possible presence of raffinose and some quite other source of 'glucose' is indicated.

8. Estimates of sucrose obtained either as the increase of reducing sugar after the action of invertase for an hour or as twice the glucose produced by acid hydrolysis are therefore always too high, and estimates of fructosan as

the difference between the reducing sugar produced by acid and by invertase hydrolysis, or as the excess production of fructose over glucose by acid hydrolysis, are always too low.

9. Fructosan has been approximately estimated as the excess of fructose over glucose produced by the action of N/5 acid, plus a correction for the 6 per cent. of glucose occurring in the known fructosan. Sucrose has usually been estimated as the difference between the total increase in reducing power and the fructosan estimate. In cases where the estimate of sucrose obtained by invertase action was lower than that obtained as above the lower value was taken as the best estimate available. In the presence of large amounts of fructosan the errors on the estimates of sucrose may be of the order of 30 per cent.

10. Sampling errors of sugar determinations carried out by the above methods are given, being calculated from the differences between the estimates of sugar in duplicate samples of ten plants gathered at different dates.

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On the Nature of Competition between Plants in the Early Phases of their Development¹

BY

S. C. VARMA, PH.D.

With Plate VI and five Figures in the Text

THE experiments hereafter described were begun at the suggestion of Professor E. J. Salisbury in an attempt to determine experimentally whether competition is actually more severe between individuals making similar demands upon the habitat, i.e. between individuals of the same species or varieties, or between individuals whose biological equipment is different even if their demands be similar, at least in the initial phases of development, i.e. between individuals of different species or varieties. An attempt has also been made to find out the nature of competition and its *modus operandi* in the particular cases investigated.

I. THE SEVERITY OF COMPETITION BETWEEN DIFFERENT KINDS OF PLANTS

The percentage death-rate can with due precaution be utilized as a measure of the severity of competition among plants, a higher death-rate indicating more severe competition. The death-rate of a large variety of plants was determined in pure cultures and in mixed cultures consisting of more than one variety of plant. The cultures were put up in glazed earthenware pots and in each experiment they were arranged as follows:

- Pot 1. 100 seeds of species A. 0.25 cm. apart.
- „ 2. 50 seeds of species A and 50 seeds of species B. 0.25 cm. apart.
- „ 3. 100 seeds of species B. 0.25 cm. apart.
- „ 4. 100 seeds of species A. 0.5 cm. apart.
- „ 5. 50 seeds of species A and 50 seeds of species B. 0.5 cm. apart.
- „ 6. 100 seeds of species B. 0.5 cm. apart.

The seeds when sown were carefully placed at the intervals stated, and in the mixed cultures the seeds of the competing species were alternated.

In each experiment six pots were generally employed and the physical and chemical nature of the soil was approximately similar at the beginning. The water supplied was ample to meet the needs of all the plants and they were exposed to the same temperature, humidity, light intensity, and other atmospheric conditions. The growth of the plants was relatively better in pot

¹ Part of a thesis approved for the degree of Ph.D. in the University of London.

cultures than in nature, but the conditions were unfavourable for adult plants, many of which died prematurely. This aspect of the pot cultures within the greenhouse does not affect the value of these experiments in respect to the early phases of development upon the mortality during which stress is alone laid.

TABLE I

Percentage Death-rate of Plants in Pure and Mixed Cultures

Shirley poppies: A (Scarlet-edged white); B (Flesh); C (White); E (Salmon); D *Papaver hybridum*; F (Orange-scarlet).

Weeks:	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.
A alone	—	—	—	—	5.4	9.6	20.7	24.2	56	45.4	33.3	25	0	33.3	100
A and B	—	5.8	0	0	6.2	6.6	14.2	50	16.6	20	25	33.3	50	0	100
A alone	—	—	—	2.4	2.5	0	7.6	22.2	42.8	25	83.3	100	—	—	—
A and C	—	—	—	—	—	—	—	—	10	16	33.3	60	0	100	—
D and E	—	—	—	—	—	—	2.1	62.2	41.1	40	83.3	100	—	—	—
D alone	—	—	2.1	0	1	2	20.2	80.2	85.7	50	100	—	—	—	—
C	25	8.3	30	100	—	—	—	—	—	—	—	—	—	—	—
C and F	—	41.4	33.3	81.2	33.3	50	0	0	0	100	—	—	—	—	—
C alone	—	3.5	1.6	0	0	5	7.1	7.6	27	37.1	77.2	80	100	—	—
C and A	—	5.5	0	0	6.3	0	0	9	25	20	25	22.2	42.8	100	—

Papaver hybridum and *P. argemone*.

Weeks:	15.	16.	17.	18.	19.	20.	21.	22.	23.	27.	28.	30.
<i>P. hyb.</i>	—	—	—	—	—	—	—	—	—	—	—	—
<i>P. hyb.</i> and <i>P. argem.</i>	10.5	5.8	6.2	0	0	0	60	0	33	50	50	100
	—	—	25	0	0	0	100	—	—	—	—	—

Hypericum montanum alone and with *H. pulchrum*.

Weeks:	7.	8.	9.	10.	12.	13.	15.	16.	17.	18.	19.	22.	23.	34.	36.	37.	38.	39.	40.
Alone	2	2	1	2	2	3.2	8.9	4.9	7.7	4.1	1.4	4.3	4.5	3.2	11.6	5.6	8	4.3	4.5
With <i>H. pulchrum</i>	2	2	0	0	0	2	14.8	5	0	0	0	7.8	11.4	3.2	3.3	6.8	0	0	3.7

Hypericum montanum alone and with *H. perforatum*.

Weeks:	4.	5.	6.	7.	8.	9.	11.	14.	15.	16.	17.	18.	19.	27.	29.
Alone	—	—	—	—	1.6	0	—	3.3	1.7	1.7	3.5	14.8	2.1	2.2	6.8
Mixed	—	2.7	5.6	3.0	3.1	3.3	—	—	—	6.8	14.8	—	—	8.6	—

Weeks:	30.	31.	32.	33.	34.	35.	36.	37.	38.	39.	40.
Alone	5	2.6	27	3.7	30.7	22	42.8	12.5	42.8	75	100
Mixed	4.7	—	30	—	42.8	25	16.6	100	—	—	—

Hypericum pulchrum alone and with *H. perforatum*.

Weeks:	8.	9.	10.	11.	12.	13.	23.	25.	26.	27.	28.	29.	30.
Alone	—	1.2	2.6	4	1.4	4.2	4.2	0	0	2.9	0	3	9.5
Mixed	5.5	11.7	20	12.5	9.5	0	10.5	11.7	2.0	25	11.1	25	100

Silene noctiflora (A) and *S. pendula* (B).

Weeks:	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	19.	20.	21.
A pure	5.7	25	—	—	—	—	—	3	—	1.5	7.9	—	—	—	—	—	—	—	—
A mixed	—	—	—	6	—	—	—	36.9	3.4	21.4	9	—	—	—	—	—	—	—	—
B pure	—	16.6	9.3	—	—	2.9	3	—	—	—	—	—	—	—	—	—	—	—	—
B mixed	—	6	6.5	—	2.3	4.7	2.5	—	71.5	—	27.2	—	—	—	—	—	—	—	—
A pure	5.6	—	—	—	—	—	—	—	1.2	9.6	32	11.6	21.4	18.1	33.3	50	55.5	100	—
A mixed	—	—	—	2	—	—	—	2	2	4.2	2.2	11.3	15.6	12.1	29.5	33.3	43	50	100
B pure	—	16.1	—	—	—	—	1	18.1	22.2	6.1	10.8	48.7	52.3	100	—	—	—	—	—
B mixed	—	—	—	5.8	—	—	—	—	3.1	—	35.4	65	42.8	100	—	—	—	—	—

Silene quadrifida alone and with *S. alpestris*.

Weeks:	4.	5.	6.	9.	10.	11.	12.	14.	22.	25.	29.	30.	31.
Pure	2.8	2.8	8.8	9.3	3.4	14.2	8.3	4.5	4.7	21	13.3	23	—
Mixed	—	—	—	8.3	18.1	11.1	—	—	—	—	—	—	12.5

Weeks:	32.	34.	36.	37.	40.	45.	46.	47.
Pure	—	—	10	11.1	37.3	—	46	66.6
Mixed	—	—	14.2	—	20	50	—	100

The percentage death-rate per week of the various plants in pure cultures and in mixed cultures is shown in the graphs where the ordinates represent the death-rate as a percentage of the individuals alive at the beginning of any given week and the age in weeks is shown by the abscissa (cf. Table I and Text-figs. 1-5).

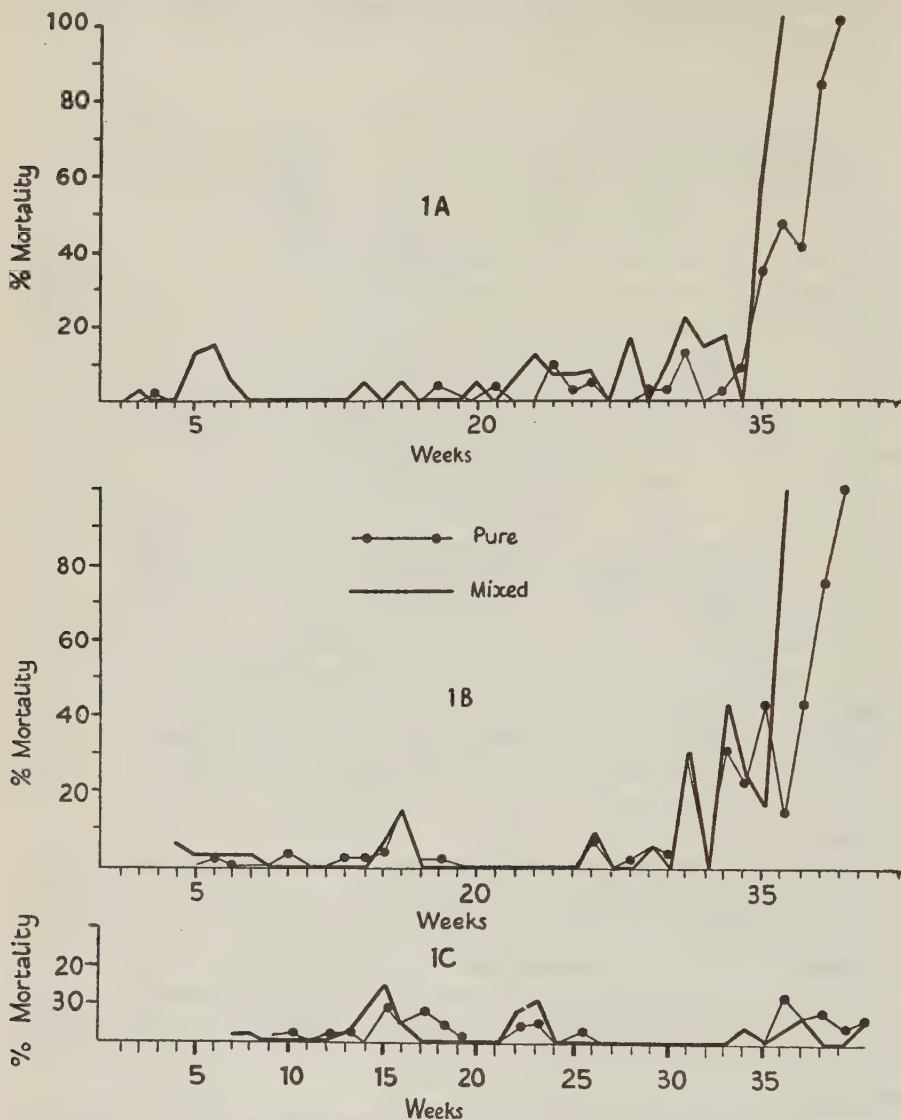
It has been held by Darwin that severity of competition between plants belonging to the same species, because of the similarity of their demands, is more severe than between individuals of two distinct species or varieties. The results of these experiments do not show this hypothesis as generally true. It is found that in a number of instances the severity of competition is greater when two different, but ecologically allied, species are competing than when the competition is between individuals of the same species.

Thus Darwin's generalization is not always valid. Poppy A (White Shirley with a scarlet edge) has been found to show earlier mortality in pure culture than when in competition with a pure white strain (Text-fig. 2), whereas in competition with a flesh-coloured variety (Text-fig. 5) the death-rate was higher in the early phases of the mixed cultures than in the pure. *Papaver hybridum* (cf. Text-fig. 5) showed an earlier mortality in the pure culture than when in competition with Shirley Poppy (var. Salmon-rose), but the juvenile death-rate was higher when competing with *Papaver argemone* than when the competition was between individuals of *P. hybridum* alone. A pure white strain of Shirley Poppy showed higher death-rates in pure cultures than in mixed whether the competing variety was an orange-scarlet strain or white-edged scarlet (Text-fig. 2). The wild species *Hypericum montanum* (Text-fig. 1) showed a lower death-rate in pure culture whether the competing species were *Hypericum pulchrum* or *H. perforatum*. *H. montanum* is a local species, a feature that may be due to an intolerance of competition. Both *Hypericum pulchrum* and *H. perforatum* showed earlier mortality in mixed cultures (Text-fig. 4).

Experiments with *Silene noctiflora* and *S. pendula* v. *compacta* showed higher mortality when growing in pure stands than when competing with one another (Text-fig. 3). The hypothesis that competition becomes more and more severe with increasing similarity of plants may therefore often be false, and these experiments show that severity of competition may be greater between members of the same species under one set of conditions and between members of different species under another set of conditions.

From the examination of Text-fig. 4 it will be observed that the death-rate curve shows two peaks, one at the seedling stage and the other when the plants have reached comparative maturity. Under ordinary conditions of growth one would expect the two peaks to be sharply defined and the first to show a higher death-rate than that which is observed under control conditions; but owing to the measure of protection afforded to the seedlings under greenhouse conditions, the death of a certain percentage of them was delayed.

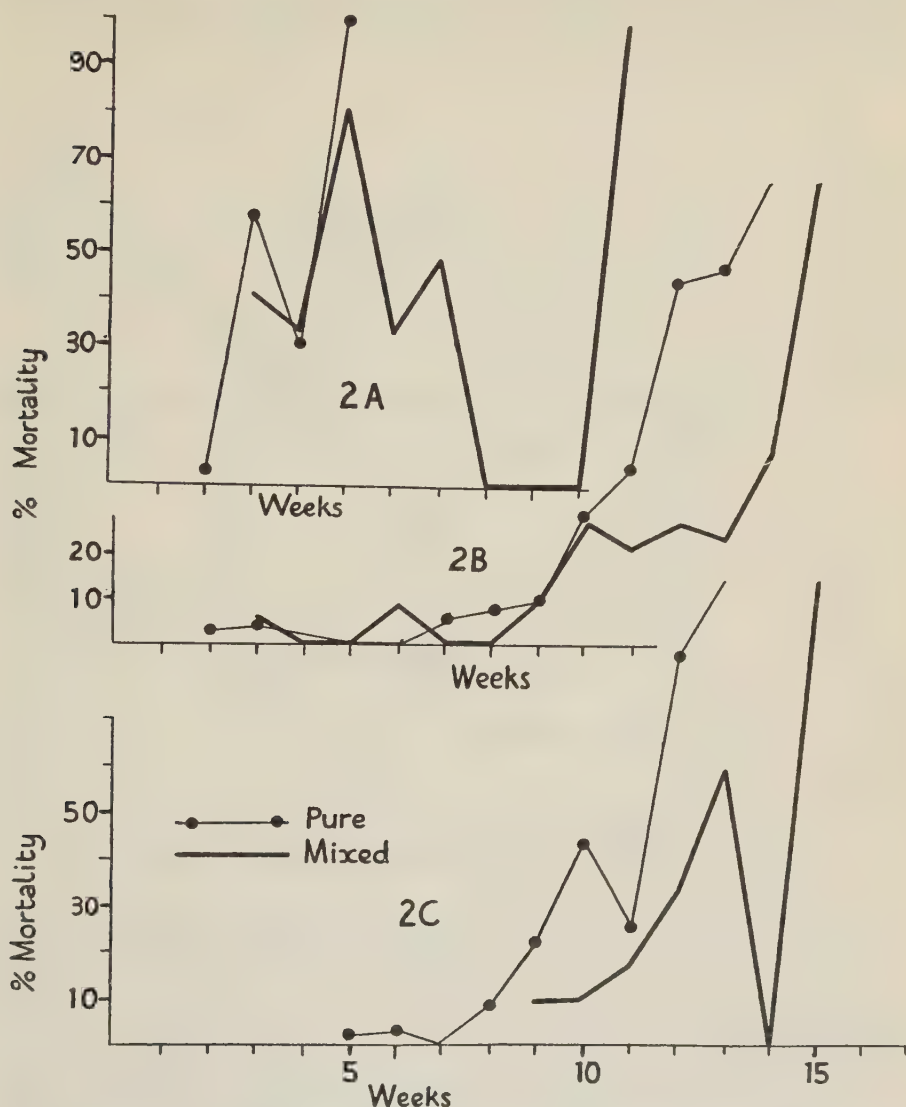
The accompanying data (Table I and Text-figs. 1-5) show the weekly deaths expressed as a percentage of the total individuals at the commencement of



TEXT-FIGS. 1 A-C. Percentage mortality plotted against time of *Hypericum montanum* growing alone or in mixed culture. 1 A. *Hypericum montanum* and *H. perforatum* 0.5 cm. apart. 1 B. 0.25 cm. apart. 1 C. *H. montanum* and *H. pulchrum*.

the week. Owing to the very unfavourable conditions of growth in the later stages, little, if any, importance attaches to the actual magnitude of the mortalities at this phase, but the data have a value from the differential effect of

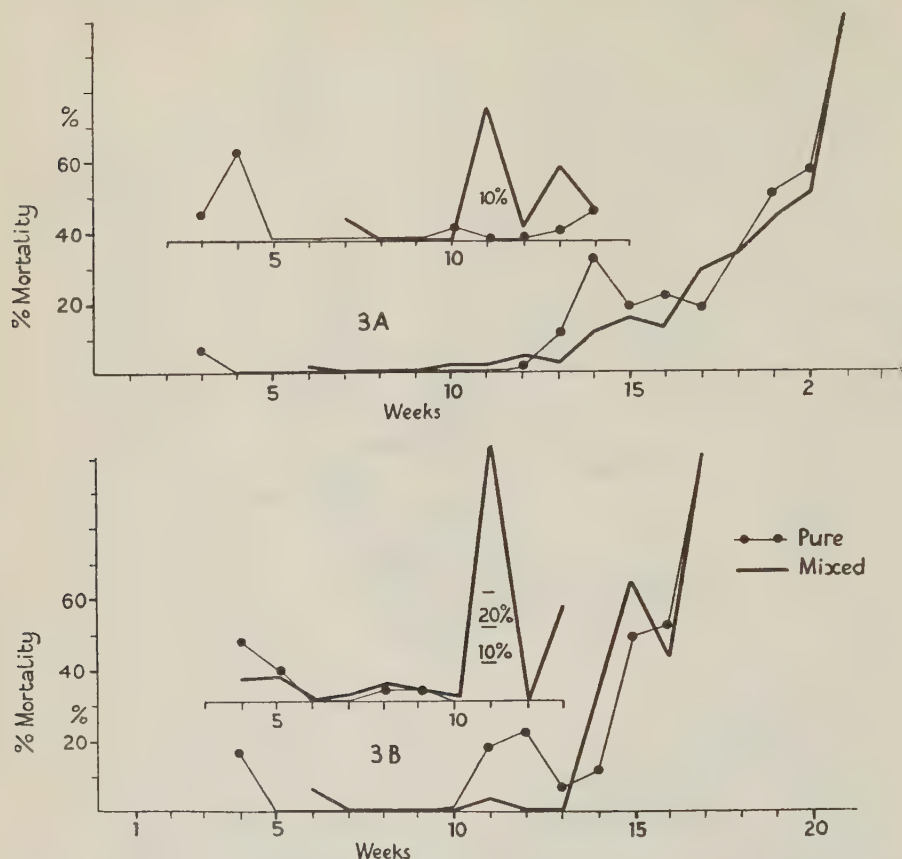
these unfavourable conditions on the pure and mixed cultures and on the respective species. It should be noted that a high percentage mortality in the



TEXT-FIGS. 2 A-C. Percentage mortality plotted against time for various plants growing alone or in mixed culture. 2 A and 2 B. Mortality of white Shirley poppy in competition respectively with white-edged scarlet and orange poppies. 2 C. mortality of white-edged scarlet in competition with pure white poppy.

later stages usually represents a very much smaller number of deaths than the same percentage in the earlier part of the life-history. Severity of competition is probably greatest in the early seedling stages, and, as the curves show,

may practically cease. The second peak represents the onset of the unfavourable conditions referred to which, since it occurred even in the perennial species, may be safely attributed to the fact that the volume of soil was too



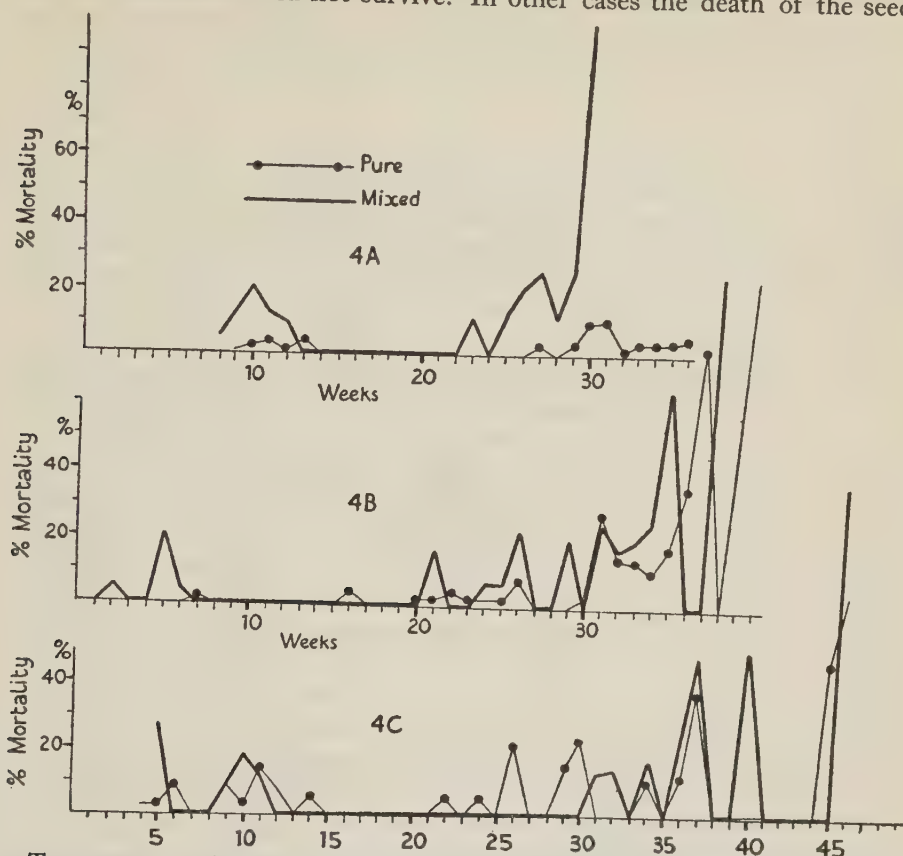
TEXT-FIGS. 3 A and B. Percentage mortality plotted against time for *Silene noctiflora* (3 A) and *S. pendula compacta* (3 B) growing alone or in mixed culture.

restricted for development beyond the early stages for the study of which the experiments were initiated.

It may therefore be suggested that there is no competition between closely aggregated seeds, but they begin to show signs of competition just after germination. The severity of competition increases with the growth and increasing differentiation of the seedlings only up to a certain stage when the death-rate reaches a maximum, after which the competition gradually decreases. But as the plants attain maturity competition may again be severe, though perhaps much less selective in character.

The causes of the high mortality at the seedling stage may be various. It has been observed in an experiment that one plant of poppy (Improved

Shirley, orange-scarlet) out of ninety-seven that germinated did not develop chlorophyll and died on the third day after germination. Of the ninety-eight seeds of another poppy that germinated (Improved Shirley, pure white) two were albinos and could not survive. In other cases the death of the seed-

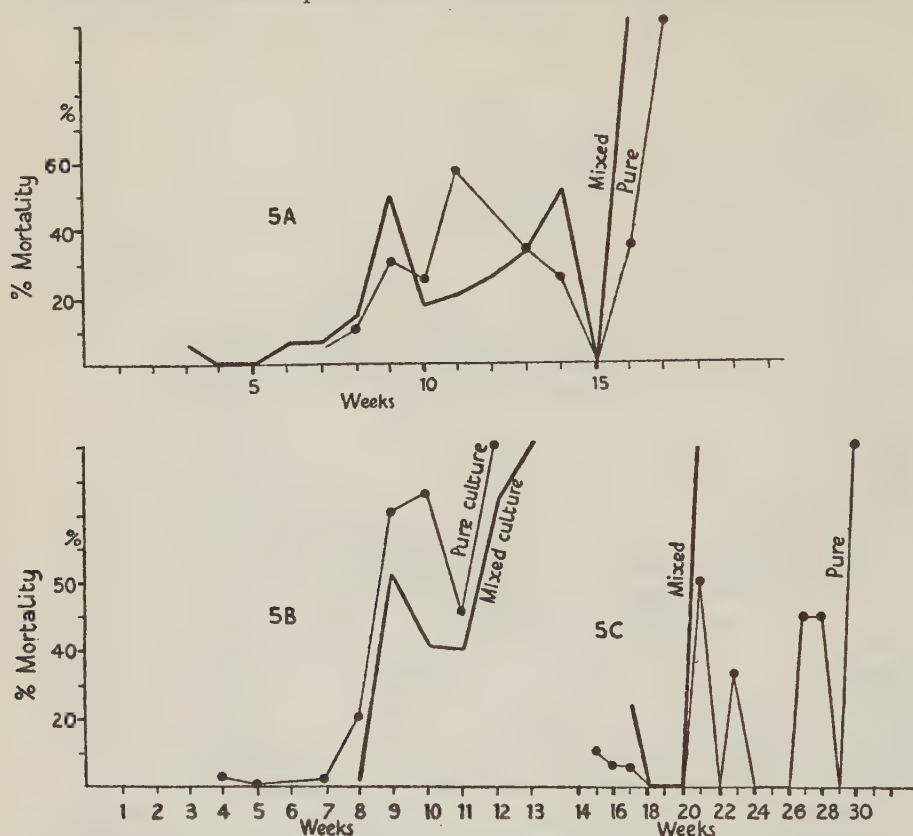


TEXT-FIGS. 4 A-C. Percentage mortality plotted against time. 4 A. mortality of *Hypericum pulchrum* alone and with *H. perforatum*, 0.25 cm. apart; 4 B. of *H. perforatum* alone and with *H. montanum*, 0.5 cm. apart; 4 C. of *Silene quadrifolia* alone and with *S. alpestris* 0.5 cm. apart.

lings could be ascribed to the premature drying up of their cotyledonary leaves, or their inability to remain erect, or else to some unknown physiological cause. Only those plants survived which by virtue of their physiological and morphological equipment escape death under the particular conditions. Out of the many causes that may be assigned for the death of the seedlings at the early age, the possible production of toxic substances by roots has been given special attention and the results are discussed in the next section.

It may, perhaps, be suggested that tolerance of competition by individuals of the same taxonomic category may depend on the degree of physiological differentiation within the race. The more uniform the individuals of a particular strain the more likely they are to persist in a balanced though

impoverished condition. With greater differentiation within the race there is increased probability of survival of some individuals over others. At one extreme are species whose demands are sufficiently complementary to reduce their mutual competition to a minimum. At the other extreme are



TEXT-FIGS. 5 A-C. Percentage mortality plotted against time. 5 A. of *Papaver Shirley* 'white scarlet edged' alone and with Shirley 'flesh.' 5 B and 5 C. mortality of *Papaver hybridum* alone and respectively with Shirley salmon-rose and *P. argemone* 0.25 cm. apart.

species whose mutual demands and equipment are so similar that a balanced and impoverished *modus vivendi* is attained, while it is between these two extremes that competition resulting in elimination of individuals is most severe.

These three categories cannot be sharply delimited, and it appears that competition gradually becomes more severe with the increasing differences between the competing individuals only up to a certain stage when the competition reaches a maximum. After this stage is reached the competition may be gradually reduced since the associated species may through differences in root and shoot development make demands on different soil and air regions. It is, however, doubtful whether species are, in nature, ever completely complementary (Watt and Fraser 1933).

II. THE POSSIBLE PRODUCTION OF TOXIC SUBSTANCES BY THE ROOTS OF PLANTS

Spencer Pickering and the Duke of Bedford (1919) have claimed to demonstrate by means of experiments carried out at the Woburn Experimental Fruit Farm that living roots of plants produce toxic substances in the soil and that these affect adversely the growth of other plants in the vicinity. This claim still needs confirmation, because such an action of roots is denied by others.

The two authors used plants growing in perforated iron or earthenware trays which were placed over the pots for demonstrating the action of toxins. In the author's experiments described below glass funnels were used instead to eliminate the possibility of contamination of the solution through contact with metal surfaces. In every experiment six funnels and nine glazed earthenware pots were used as a rule. They were arranged as shown in Pl. VI, Fig. 1. The six funnels were supported on a wooden rack and a pot placed underneath each funnel. Three pots were placed in the front row and, receiving no leachings from the funnels, served as controls.

In each experiment two species of plants (say A and B) were used. In two funnels the seeds of species A were sown and in two others the seeds of species B. The other two funnels had no seeds and served as controls; these are referred to in the following description as 'dummy funnels'. Three out of the six pots under the funnels contained seeds of species A and three of species B. Two of the three control pots shown in the front row contained pure cultures of either species A or of species B, and the third one a mixed culture of species A and B.

The funnels and pots were so arranged that out of the three pots containing species A the first one received leachings from a funnel without plants (dummy funnel), the second one received leachings from the funnel containing species A, and the third one from the funnel containing species B. Similarly, out of the three pots containing species B the first one received leachings from the dummy funnel, the second one from the funnel containing species A, and the third one from the funnel containing species B. Thus in each set of three pots containing either species A or B one pot received leachings from soil alone, while the others received leachings from the soil together with any soluble substances produced by the same or a different species of plants. For the sake of convenience these experiments will be referred to in the subsequent description as 'Funnel experiments'.

These experiments were repeated a number of times, and the amount of dry matter formed by 100 shoots is shown in Table II. It will be seen that the growth, as measured by dry weight, in plants receiving leachings from a different species of plant was less than that of plants receiving leachings from the same species. The amount of dry matter formed was highest in plants grown under the dummy funnels, thus indicating that the leachings from funnels containing plants of either the same or different species had an

adverse effect on the growth of plants in the pots. The plants grown in mixed culture showed less dry weight than those grown in pure culture.

The poorer growth of *Brassica alba* and *B. oleracea* under funnels containing plants than under dummy funnels containing soil but no plants

TABLE II
Leaching Experiments with Brassica

Plant in pot.	Plant in funnel.	Dry weight of 100 shoots (gm.).				
		Exp. 8b.	Exp. 8c.	Exp. 15.	Exp. 17.	Exp. 19.
<i>B. alba</i>	None	1.67	1.23	1.64	1.72	0.75
"	<i>B. oleracea</i>	1.43	—	1.42	1.56	0.60
"	<i>B. alba</i>	—	1.27	1.67	1.65	0.71
"	(Pure culture)	—	—	1.90	1.33	0.95
"	(Mixed culture)	—	—	1.85	1.27	0.80
<i>B. oleracea</i>	None	1.40	1.31	1.65	1.68	1.16
"	<i>B. alba</i>	0.98	—	1.40	1.16	1.11
"	<i>B. oleracea</i>	—	1.32	1.59	1.28	1.07
"	(Pure culture)	—	—	1.89	1.56	1.30
"	(Mixed culture)	—	—	1.18	1.15	1.16

seems to be due to the presence of some deleterious substance in the leachings from the funnels. The deleterious substance affected more adversely individuals of another species than of that producing it. The poorer growth of plants in the mixed than in the pure cultures also seems to be due to the presence of some deleterious substance produced by the roots of plants.

Silene noctiflora and *S. pendula-compacta* showed higher death-rate in pure cultures than in mixed cultures (vide Table I, Text-fig. 3). The competition was therefore more severe among individuals of the same species. This is confirmed by the higher dry weight of these plants in mixed than in pure cultures. Funnel experiments with these plants also gave similar results as shown in Table III. It is possible that the toxins that might have been produced by the roots, instead of retarding the growth of a different species, have in fact stimulated them to such an extent that they showed better growth in mixed than in pure cultures.

Table IV shows the results of three funnel experiments with *Brassica alba* and *B. oleracea*. The shoots of the plants in the funnels were removed after a couple of weeks, leaving the roots of the plants in the soil, and the pots underneath received leachings from decaying roots. The results are consistent and showed that the toxic effect was accentuated by the removal of shoots. These results, therefore, support the hypothesis of the workers in the U.S. Department of Agriculture that the toxic substances are probably a product of the decay of roots, or of the cells that are continually being lost by the growing roots.

These results also show that the toxic substances act in aqueous solution, and they can be washed away. It is probable, therefore, that the plants grown in soils frequently leached should show better growth than those grown under conditions where leaching does not occur, provided the soil nutrients are

regularly replaced. The results of experiments 61, 64, 100, and 101 are shown in Table V. In each experiment four funnels were taken each containing 200 gm. of washed silver sand. Two of these funnels contained pure cultures

TABLE III
Leaching Experiment

Plant in pot.	Plant in funnel.	Dry wt. of 100 shoots (gm.).		
		Exp. 20.	Exp. 68.	Exp. 83.
<i>S. noctiflora</i>	None	0.38	0.24	0.32
"	<i>S. pendula-compacta</i>	0.40	0.307	0.32
"	<i>S. noctiflora</i>	0.28	0.402	0.31
" (Pure culture)	No funnel	0.52	0.21	0.42
" (Mixed culture)	"	0.57	0.28	0.50
<i>S. pendula-compacta</i>	None	0.36	0.37	0.31
"	<i>S. noctiflora</i>	0.26	0.39	0.44
"	<i>S. pendula-compacta</i>	0.35	0.22	0.28
" (Pure culture)	No funnel	0.42	0.33	0.36
<i>S. pendula-compacta</i> (Mixed culture)	"	0.27	0.34	0.37

TABLE IV
Leaching Experiment with Brassica

Plant in pot.	Plant in funnel. Shoots removed.	Dry wt. of 100 shoots (gm.).		
		Exp. 80.	Exp. 81.	Exp. 98.
<i>B. alba</i>	No plants	1.10	1.11	1.09
"	<i>B. oleracea</i>	0.94	0.92	0.83
"	<i>B. alba</i>	1.01	1.06	1.22
" (Pure culture)		1.55	1.27	1.28
" (Mixed culture)		1.15	1.24	1.06
<i>B. oleracea</i>	No plants	1.28	1.58	1.61
"	<i>B. alba</i>	0.97	1.06	1.30
"	<i>B. oleracea</i>	1.10	1.76	1.41
" (Pure culture)		1.29	1.53	1.67
" (Mixed culture)		0.78	1.01	1.48

TABLE V

Species.	Nature of culture.	Dry wt. of 100 shoots (gm.).			
		Exp. 61.	Exp. 64.	Exp. 100.	Exp. 101.
<i>Brassica alba</i>	Pure. Not leached	5.89	1.79	0.605	1.77
" " }	Mixed. Not leached {	5.18	2.74	0.52	1.61
" <i>oleracea</i> }		7.20	2.56	0.73	1.52
" <i>alba</i> }	Mixed. Leached {	7.54	4.60	0.57	2.76
" <i>oleracea</i> }		7.56	2.75	0.69	1.67
" " }	Pure. Not leached	7.92	3.31	0.85	1.68

of either *Brassica alba* or *B. oleracea* and the other two mixed cultures of the two species. One of the funnels containing mixed cultures was subjected to leaching by adding an excess of 0.14 per cent. normal Knop's solution, so that the solution flowed out by the spout. Other funnels also received the same solution, but the addition of the solution was stopped as soon as a drop appeared in the spout, thus ensuring that the soil in all the funnels was equally moist. This process was repeated every morning.

It will be seen from the results in Table V that the growth in mixed leached cultures was much better than in mixed unleached cultures. The growth of plants in mixed unleached cultures was poorer than in the pure unleached cultures (Pl. VI, Fig. 2).

The repetition of the above experiments with the seeds of *Silene noctiflora* and *S. pendula-compacta*, of which the results are shown in Table VI,

TABLE VI

Species.	Nature of culture.	Dry wt. of 100 shoots (gm.).
		Exp. 72.
<i>Silene noctiflora</i>	Pure culture. Not leached	0.95
" " <i>pendula-compacta</i> }	Mixed culture. Not leached {	1.23
		1.01
" <i>noctiflora</i> }	Mixed culture. Leached {	1.47
		1.12
" <i>pendula-compacta</i> }		1.03
" " "	Pure culture. Not leached	

showed that the growth was better in the mixed leached funnel than in the unleached mixed culture. The growth of plants in mixed unleached culture was better than in unleached pure culture.

The better growth of plants in leached cultures seems to be due to the removal, by leaching, of the toxins produced by the roots. *Brassica alba*, though growing in mixed culture, showed better growth than those growing in unleached pure culture, thereby indicating that the toxins adversely affect the plant producing them. The toxins seem to affect more adversely individuals of a different species than individuals of the species producing it. This is indicated by the poorer growth of plants in the unleached mixed culture than in the unleached pure culture. Pl. VI, Fig. 2, shows the growth of plants in different funnels of experiment 61.

It is well known that for good growth of plants in water cultures the nutrient solution should be changed frequently and, if possible, continuously (Allison and Shive, 1923). It is believed that the culture solution is rendered unsuitable after a few days because of deficient oxygen and increase of carbonic acid or because of the disturbance of the physiological balance of the solution. It has been pointed out by Miller (1931) that the organic compounds produced by the root-cells that are lost by the growing root-tip may also have a detrimental effect upon the growth of plants. In a water-culture experiment with *B. alba* and *B. oleracea* in pure and mixed cultures, it has been found that *B. alba* showed better growth in pure than in mixed culture, while *B. oleracea* showed better growth in mixed than in pure culture. Further experiments are, however, needed to elucidate the factors involved, but it is possible that some toxic action, resulting from the decay of the root-cells, as suggested by Miller, might be responsible for such results.

These results as a whole indicate that there is some deleterious substance produced in the soil by the presence of roots. This is in accordance with the

results obtained at Woburn Experimental Fruit Farm by Pickering and the Duke of Bedford (1919). The latter, however, believed that only living roots produced these poisonous substances, but in the experiments described above the deleterious effects were not only in evidence when the plants were living but also when the roots were decaying after the shoots were removed. In the latter case the effect of the deleterious substances was more marked, and therefore these results, as pointed out before, support to a certain extent the hypothesis of the workers in the U.S. Department of Agriculture.

It also appears that when two species are growing in mixed cultures the toxic substances produced by one of them may be more harmful to the individuals of the other species than they are to the individuals of the same species. The selective action of these toxins seems to provide each competing species with a 'weapon' (Warming, 1909) by which it depresses the vigour of its competitors, thus exerting a profound influence upon the outcome of competition. On the other hand, in some cases (*Silene pendula-compacta* and *S. noctiflora*) the substances produced would appear to stimulate, rather than depress, the growth of competing species.

The nature of the toxic substances produced by the roots of plants is still obscure. It was believed by some older botanists, as well as Pickering and the Duke of Bedford, that they are excretions from the roots. Hansteen-Cranner has found that certain phosphatides are passed out into the soil by exosmosis from certain roots, but the relation of these phosphatides to soil toxicity is uncertain. On the other hand, some American workers believe that the poisonous substances are not excreted, but are produced by the decay of the disintegrating root-cells which are continually being lost by the growing root-tip. The poisonous substances, as pointed out above, appear to be formed by living as well as by decaying roots.

These results suggest that the soluble toxins produced by the roots of plants differ either in amount or in kind according to the species involved. The varying reaction according to the combination of species involved suggests a qualitative rather than a purely quantitative difference. It is therefore probable that different plants produce different kinds of toxins which differ from one another both in their manner of production and in their physical and chemical properties. A plant seems to be more immune from the toxins produced by itself or its own kind than from the toxins produced by a different kind of plant. The hydrogen-ion-concentrations of the leachings from different funnels of the 'funnel experiments' were almost of the same value, and therefore their differing effect was probably unrelated to soil reaction.

III. THE STRUCTURAL AND PHYSIOLOGICAL RESPONSE OF PLANTS TO THE PRESENCE OF TOXINS IN THE SOIL

The presence of toxic substances in the soil should evoke certain responses in plants growing in it. These responses may be structural or physiological. Salisbury (1932) suggested that 'the stomatal frequency of plants can with

due precaution be utilized as a phytometric index of the resultant combination of both external and internal factors, which determine the water relation of plants'. With this suggestion in view an attempt was made to find out the frequency and size of the stomata of plants growing in pure and mixed culture and also when they were receiving leachings from funnels containing the same or a different species.

The leaves of comparable heights of the plants in the pots of a 'funnel experiment' were removed and immediately killed and preserved in 95 per cent. alcohol. After these leaves were free from chlorophyll the lower epidermis was examined for the determination of stomatal frequency, the size of stomata, &c. Similarly the stomatal frequency as well as the number of epidermal cells were determined in the case of *Brassica alba* and *B. oleracea* (Table VII) as well as of *Silene pendula-compacta* and *S. noctiflora* (Table VIII).

The results of these two experiments are consistent. A higher stomatal frequency is observed in the plants grown in mixed than in those grown in pure culture. Also the frequency was higher in those that received leachings from funnels containing a different species than in those receiving leachings from a funnel without any plants. This was most probably due to a larger proportion of stomata formed from epidermal cells, as is borne out by the fact that the stomatal index shows a rise in the same sense. After the corrections for the rise of the stomatal index were made these differences became very small, thus showing that the increase in the stomatal frequency was not due to the smaller growth of the epidermal cells but to a percentage increase in the number of stomata mother-cells produced from the epidermis.

A slightly higher frequency of epidermal cells per unit area was also observed in the plants grown in mixed than in pure cultures and in those that received leachings from funnels containing a different species than in those that received leachings from the dummy funnel. These differences become more marked when the number of epidermal cells and the stomata per unit area are added together; the stomata can be considered as specialized epidermal cells. This eliminates any error that is likely to be caused if the number of epidermal cells are alone considered apart from the number of stomata produced by them. The rise in the stomatal index in plants grown in mixed as compared with those in pure culture suggests that the rise in frequency of epidermal cells in the same sense is perhaps due to the division of the epidermal cells and not due to their decrease in size.

The variation in the stomatal index, as Salisbury (1927) has pointed out, 'appears to be due to the internal factors of which nutritional conditions are perhaps the most important'. It is therefore very likely that the change in stomatal index and the number of epidermal cells indicate a changed nutritional balance of plants grown in mixed cultures.

It is possible that such a disturbance in the nutritional balance may be accompanied by a change in the suction pressure of plants. The following

TABLE VII
Experiments with Brassica

Plant in pot.	Plant in funnel.	Stomata per unit area (mean of 25 counts).	Range of stomata per unit area.	Frequency of stomata when corrected for the stomatal index of the control.	Epidermal cells per unit area (mean of 25 counts).	Range in the no. of stomatal cells per unit area.	No. of stomata plus epidermal cells per unit area.	Stomatal index.	Length of stomata (mean of 25).	Range of stomatal length.
<i>B. alba</i>	Dummy	13.2 ± 1.3	8 to 17	13.2	26.6 ± 2.1	15 to 33	39.8	33.1	7.6 ± 0.53	6 to 9
"	<i>B. oleracea</i>	16.0 ± 1.3	12 to 20	14.2	28.8 ± 2.3	23 to 34	44.8	35.7	7.0 ± 0.16	6 to 8
"	<i>B. alba</i>	15.0 ± 1.2	11 to 18	13.4	27.2 ± 2.1	23 to 36	42.2	35.5	7.0 ± 0.55	6 to 9
" (Pure culture)		12.9 ± 2.0	7 to 20	12.9	26.5 ± 3.5	16 to 38	39.4	32.7	7.8 ± 0.54	6 to 9
<i>B. alba</i> (Mixed culture)		16.6 ± 1.4	12 to 20	15.7	32.7 ± 2.6	25 to 38	49.3	33.6	6.7 ± 0.39	6 to 8
<i>B. oleracea</i>	Dummy	12.9 ± 1.2	9 to 16	12.9	27.0 ± 1.1	15 to 31	39.9	32.0	7.7 ± 0.61	6 to 10
"	<i>B. alba</i>	15.5 ± 1.4	13 to 21	14.3	30.5 ± 2.5	23 to 44	46.0	33.6	6.4 ± 0.8	4 to 10
"	<i>B. oleracea</i>	17.4 ± 1.5	13 to 23	16.4	35.0 ± 2.6	28 to 42	52.4	33.2	6.2 ± 0.54	5 to 8
" (Pure culture)		13.0 ± 1.8	7 to 18	13.0	25.6 ± 1.8	21 to 32	38.6	33.6	9.0 ± 0.69	7 to 11
<i>B. oleracea</i> (Mixed culture)		14.5 ± 1.6	9 to 19	13.8	27.3 ± 3.0	20 to 39	41.8	34.6	8.2 ± 0.66	6 to 10

TABLE VIII
Experiments with Silene

Plant in pot.	Plant in funnel.	No. of stomata per unit area (mean of 20 counts).	Range in the no. of stomata per unit area.	Frequency of stomata when corrected for stomatal index of the control.	No. of epidermal cells per unit area (mean of 20 counts).	Range in the no. of epidermal cells per unit area.	Total no. of stomata and epidermal cells per unit area.	Stomatal index.	Length of stomata (mean of 20 counts).	Range in length of stomata.
<i>S. noctiflora</i>	Dummy	7.1 ± 1.6	4 to 11	7.1	21.6 ± 6.0	6 to 38	28.7	24.7	12.2 ± 1.4	10 to 17
"	<i>S. pendula-compacta</i>	8.7 ± 2.6	4 to 16	7.1	22.3 ± 5.1	12 to 36	31.0	28.06	11.5 ± 1.1	9 to 15
"	<i>S. noctiflora</i>	7.8 ± 1.7	4 to 14	6.8	20.8 ± 5.1	9 to 34	28.6	28.30	11.3 ± 0.76	9 to 13
"	(Pure culture)	7.3 ± 2.1	4 to 13	7.3	19.7 ± 6.9	7 to 34	27.0	26.6	12.5 ± 1.8	9 to 16
<i>S. noctiflora</i> (Mixed culture)		7.6 ± 1.4	5 to 12	7.2	20.0 ± 6.0	9 to 33	27.6	27.5	11.6 ± 1.1	9 to 14
<i>S. noctiflora-compacta</i>	Dummy	5.9 ± 1.48	3 to 10	5.9	17.3 ± 4.32	8 to 27	23.2	25.4	12.5 ± 1.7	10 to 18
"	<i>S. noctiflora</i>	7.5 ± 2.17	4 to 15	6.9	20.4 ± 3.39	6 to 39	27.9	26.8	11.5 ± 1.1	9 to 15
"	<i>S. pendula-compacta</i>	5.1 ± 1.03	4 to 8	4.8	14.2 ± 3.3	8 to 24	19.3	26.4	12.1 ± 1.3	8 to 15
<i>S. pendula-compacta</i> (Pure culture)		6.4 ± 1.54	3 to 10	6.4	16.3 ± 5.8	5 to 30	22.7	28.1	12.7 ± 1.9	9 to 20
<i>S. pendula-compacta</i> (Mixed culture)		6.5 ± 1.20	4 to 11	6.5	16.5 ± 4.7	7 to 30	23.0	28.2	12.9 ± 1.4	9 to 16

methods were employed to determine differences, if any, in the suction pressure in leaves of plants grown in mixed and pure cultures.

In experiments 33, 39, 45, and 79 the suction pressure of the leaves was determined by the method described by Molz in 1926. Employing this method, a series of ten solutions of pure cane sugar in distilled water were made having molecular concentrations varying from 0.05, 0.10, 0.15 to 0.50, which were kept in glass-stoppered bottles. About 5 c.c. of the solution was transferred to the test-tube just before the experiment was to be started. The solution was used only once and a fresh solution was taken for each experiment.

The plants were grown in pure and mixed cultures in unglazed earthenware pots with perforated bottoms. These pots were each placed in a glazed earthenware pot containing tap-water or in some cases distilled water. This was done in order to ensure that the soil in each pot was equally moist. When the plants were fully grown the leaves at comparable heights on the plants were removed and immediately immersed in paraffin oil. The leaves were cut under paraffin into small strips, cutting the main veins at right angles. The lengths of these strips were measured under a low power of the microscope ($\times 10$). The paraffin oil was then quickly removed by placing the strips between folds of blotting-paper and they were then placed in the required concentration of the cane-sugar solution.

After about two hours' immersion in the sugar solution the strips were removed and mounted under cover-slip in the same solution in which they were immersed. The lengths of these strips were again measured. The osmotic pressure of the solution in which the strips showed no change of length was taken to be equal to the suction pressure of the strips. In experiment 89 the strips were weighed on a delicate balance instead of being measured. The results are shown in Table IX.

It will be noticed that almost in all cases the leaves of plants growing in mixed culture showed higher suction pressure than those of plants grown in pure culture. Of the factors that regulate the suction pressure of plants, the atmospheric humidity and the condition of soil moisture are the most important. Any decrease in the available soil moisture increases the suction pressure of the root-cells and also that of the leaves. It seems, therefore, likely that production of toxins in the soil by the roots diminishes absorption, for all the plants were exposed to similar atmospheric conditions and were kept well supplied with water.

The slight difference in the suction pressure observed in the two species grown in mixed culture shows the varying response of different species to identical conditions. A plant with a little higher suction pressure than its neighbour will obviously be in an advantageous position in the struggle for water and nutrients.

The presence of toxic substances in the soil may not only produce a difference in suction pressure but also may restrict the extent of the root system

of the competing plants. The depth and the extent of the root system and the volume of soil that it exploits is obviously an important factor in competition. The potential height and spread of a shoot system, as has been pointed out by Salisbury (1929), is a distinct advantage to the plants in overcoming competition. The growth of the overground parts depends upon the extent of the root system and the volume of soil it exploits.

TABLE IX

Experiment no.	Species.	Method.	Suction pressure of leaves (atm.).	
			Pure culture.	Mixed culture.
33	<i>Brassica alba</i>	Molz's Method	2.6	6.7
	<i>oleracea</i>	" "	6.7	8.1
33	<i>alba</i>	" "	5.3	9.6
	<i>oleracea</i>	" "	6.7	8.1
39	<i>Silene noctiflora</i>	" "	2.6	6.7
	<i>pendula-compacta</i>	" "	1.3	4.0
45	<i>gigantea</i>	" "	5.3	8.1
	<i>critica</i>	" "	6.7	6.7
79	<i>Brassica alba</i>	" "	5.3	11.1
	<i>oleracea</i>	" "	8.1	12.7
89	<i>alba</i>	Weight Method	6.7	11.1
	<i>oleracea</i>	" "	8.1	11.1

It is, however, not easy to find out with any approach to precision the volume of soil the roots exploit, but some idea of this can be obtained by determining the total length of all the roots and rootlets of a plant. The roots of plants were separated out by immersing the pots in tap-water contained in a basin, and by carefully disentangling them under water with as little breakage as possible. These root systems were then spread out on a clean plate of glass and the length of the roots and the rootlets were measured by the help of centimetre graph paper placed underneath the plate.

In this way the root extent of *Silene noctiflora* and *S. pendula-compacta* grown as in 'funnel experiments' was determined. The results are shown in Table X. The differences between the extent of root systems between plants growing under different conditions are not statistically significant in view of the size of the probable error. It may, however, be noted that the root extent was smaller in mixed than in pure culture.

The measurements of root extent of *Brassica alba* and *B. oleracea* grown in pure and mixed cultures are shown in Table XI. The difference between the extent of root systems of *B. oleracea* grown in pure and mixed cultures was more than three times the probable error and was therefore statistically significant. In the case of *B. alba* the difference was appreciable but not statistically significant. The results indicate that the root extent of plants was smaller when they were grown in mixed than in pure cultures. In the case of *B. alba* it may be emphasized that the length of the main tap root was shorter in mixed than in pure cultures. This is shown by the last

column of Table XI. There were profuse laterals which it is probable compensated to a great extent for the poor development of the main tap root.

The extent of the root systems in the case of *Brassica alba* and *B. oleracea* as well as *Silene noctiflora* and *S. pendula-compacta* appears to be less developed

TABLE X
Leaching Experiments with Silene

Experiment no.	Plants in pot.	Plants in funnel.	Average extent of root systems (10 roots) (cm.).
39b	<i>S. noctiflora</i>	No plants	7.48 ± 0.83
27.9.1933	"	<i>S. pendula-compacta</i>	6.47 ± 0.96
	"	<i>S. noctiflora</i>	6.21 ± 0.89
	"	(Pure culture)	9.78 ± 0.69
	"	(Mixed culture)	7.96 ± 0.91
	<i>S. pendula-compacta</i>	No plants	15.05 ± 1.99
	" "	<i>S. noctiflora</i>	15.54 ± 1.99
	" "	<i>S. pendula-compacta</i>	11.19 ± 2.21
	" "	(Pure culture)	15.36 ± 2.2
	" "	(Mixed culture)	15.03 ± 2.8

TABLE XI
Experiment 63 (April 9, 1934)

Species.	Kind of culture.	Average extent of root system (10 plants) (cm.).	Average length of tap root (10 plants) (cm.).
<i>B. alba</i>	Pure	34.43 ± 6.7	11.41 ± 2.23
"	Mixed	28.22 ± 7.2	1.98 ± 0.50
<i>B. oleracea</i>	Pure	69.72 ± 9.9	12.95 ± 2.09
"	Mixed	32.47 ± 6.0	5.97 ± 1.7

in mixed than in pure cultures. There was an equal amount of nutrient material in each pot at the beginning of the experiment and all of them were well supplied with water; therefore the smaller root extent in mixed than in pure culture was most probably due to the selective effect of toxins present in the soil.

Taken as a whole, these results show that when two species or varieties which make similar demands upon the soil are grown together the resources of each of them are more severely taxed than if they were growing in pure stands. The existence of plants under such conditions is made possible by their developing features which enable them to minimize the unfavourable conditions. Those plants which cannot respond to the changed conditions will tend to disappear.

It has been shown in previous experiments that *Silene pendula-compacta* and *S. noctiflora* show better growth in mixed than in pure cultures. On the other hand, it has been shown that *Silene noctiflora* and *S. pendula-compacta* resemble *Brassica alba* and *B. oleracea* in showing higher stomatal indices and higher suction pressure in mixed than in pure culture. But *Brassica alba* and

B. oleracea differ from them in showing better growth in pure than in mixed culture.

The higher suction pressure and higher stomatal indices in *Silene noctiflora* and *S. pendula-compacta* grown in mixed compared with pure culture tend to indicate that their better growth in mixed than in pure culture was not due to the absence of toxins in the soil, but most probably to the greater capacity of these plants to withstand these toxins. The toxins have acted as a stimulus rather than as a check. The explanation for the better growth of *Silene noctiflora* and *S. pendula-compacta* in mixed than in pure culture may also be applicable to other plants which have been observed to show better growth in mixed communities in nature.

IV. DISCUSSION

It has been shown that the view that the severity of competition between plants increases with the increasing similarity of the competing members is not supported by the experiments. Certain plants survived better in pure than in mixed cultures, while others flourished better in mixed than in pure cultures. The behaviour of the same plant changed when competing with different species.

Pure communities of plants are not uncommon in nature and some of them, especially those which propagate vegetatively, are even capable of spreading rapidly. The members of a clone forming a pure community are closely alike in their morphological and physiological equipment. Their aggressiveness may in part be due to the reduced competition between the members composing the community.

Mixed communities are, however, more common in nature than pure communities. Some plants have been observed to flourish better in mixed than in pure cultures, and such plants will show a tendency to grow in mixed communities. Mixed communities may in part be due to the invasion by one species of a locality occupied by another species, neither of them being able to entirely suppress the other. A mixed community may also result when a bare area is invaded simultaneously by several species.

In the development of a plant community, e.g. Plant Succession on a bare area, competition is absent between the migrants (invaders) so long as they are distantly placed and the necessities of life are ample for each of them. After the migrants become established, and when they make increased demands on the available supplies, they will begin to compete. The competition will probably be severest between individuals making similar demands but differing in their biological equipment. The seedlings of all the plants compete, at least for a while, on equal terms and their survival will depend upon their capacity for adaptation. Afterwards all the plants that have survived competition will tend to attain equilibrium resulting in a climax plant community.

The stage at which the highest death-rate occurs can be considered as the

most critical period in the life of the plants. At this stage if one of the competitors has even a slight advantage over the others in the struggle for the necessities of life the battle may be decided in its favour. Plants are especially plastic in their seedling stage, but of differing adaptability. The different death-rate observed in the case of different species growing in the same pot demonstrates the fact that, when plants are competing, their individual capacities are tested and all of them are not equally susceptible to the same adverse factor. It is by virtue of their varying degrees of adaptability that they escape or minimize competition. Some plants may not survive simply because of their insufficient power of adaptation. A plant may be occupying a dominant position by sheer priority (Yapp, 1925) or by showing better adaptation to the particular conditions than its competitors. The dominance, as Salisbury (1929) has pointed out, 'may then be the consequence of unfavourable conditions acting by selective depression or to favourable conditions acting as a selective stimulus, but in either case the dominance is determined by the relative vigour of the species and its competitors'.

It can be scarcely doubtful that most of the plants that compete simultaneously for the same place perish, and only those survive which are best adapted for that environment. These successful plants are themselves liable to extermination if there be invasion by other plants which are better adapted. It may be emphasized that a plant is less resistant to the unfavourable conditions at the time of colonization than when established. After a time, when its root and shoot systems are well developed it has 'priority' with respect to the new invaders. In other words, the former, in the time gained by invading early, has so much strengthened its biological equipment that even though the new invader may be better fitted it is not capable of establishing itself.

The status of a plant in a community is determined by the result of the interaction of its internal constitution and the external environment which is operative at the time of its arrival.

Almost all the plants have to pass through a stringent test of natural selection before they become established at any place. There is no paradise for plants and, as Salisbury (1929) has pointed out, 'plants grow not where they would but rather where they must'.

A number of botanists have emphasized the importance of competition in the geographical distribution of plants. It has been suggested above that competition is more severe between members of different species which make similar demands than between members of the same species. Those plants adversely affected by the proximity of others in a locality will tend to form pure communities in places comparatively free from competition. Such plants when they migrate will again tend to form pure communities. If, on the other hand, they are extremely intolerant of interspecific competition they will remain isolated and restricted in range.

The plants which are not adversely affected but stimulated when in competition with other species will, on the other hand, readily migrate and will

tend to form mixed communities. The dispersal of propagules of plants is governed by external factors, but their establishment when they have reached a certain locality will depend upon the capacity of the plants to adapt themselves to that particular environment.

A higher plant by the production of toxic substances by its root, by the depletion of the nutrient material, by shading, &c., creates a special type of environment about itself, which may be termed its own biotic environment. Such an environment will be found wherever plants exist, and the presence of other plants in the vicinity will depend upon their capacity for adaptation to this particular environment or their capacity to modify it. Therefore a plant competes with another indirectly, i.e. it changes the environment immediately surrounding it, and this environment directly affects the plants with which it is competing.

Adaptation may be transitory or else may last the lifetime of the individual. It is also known that in certain cases the influence of modification may extend to subsequent generations as a diminishing 'after-effect'. Turesson (1922) has emphasized the importance of genotypic adaptation. He has shown that biotypes collected from different localities differ in their genotypical constitution, and he believes that ecotypes arise as a result of the genotypical response of an ecospecies to a particular habitat. Therefore, according to Turesson, plants show a genotypical response to a particular habitat. If such a genotypical response can be evoked by competition, then the competing plants may well be considered in the process of forming new species. Competition, as Warming (1909) has pointed out, may be producing new species.

V. SUMMARY

Experiments are described in which various species were grown in two densities, in pure and in mixed cultures. The results indicate that judged by the mortality rates the intensity of competition is usually greater in mixed cultures than in cultures of individuals of a single species. Nevertheless, the evidence afforded suggests that severity of competition may in certain cases be as great or greater between the individuals of the same species. Thus Darwin's generalization may be applicable to certain species though not to others.

The depressing effect of one species upon another is shown to be, in part, at least, due to soluble toxic substances probably formed by the roots. Evidence is furnished that these soluble substances cause an augmentation of the *suction force* and depress the root development.

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EXPLANATION OF PLATE VI

Illustrating Dr. S. C. Varma's paper 'On the Nature of Competition between Plants in the Early Phases of their Development'.

Fig. 1. Photograph showing the arrangement of funnels and pots in the 'funnel' experiments. The six pots by the rack were placed during the experiment beneath the funnels from which they received leachings.

Fig. 2. A closer view of the funnels in the 'leaching' experiment. Left to right: (i) unleached culture of *Brassica alba*; (ii) mixed unleached culture of *B. alba* and *B. oleracea*; (iii) mixed leached culture of *B. alba* and *B. oleracea*; (iv) unleached culture of *B. oleracea*.

Fig. 3. Pure and mixed cultures of Brassica. Left, pure culture of *B. alba*; middle, mixed culture of *B. alba* and *B. oleracea*; right, pure culture of *B. oleracea*.

Fig. 4. Plants grown with leachings from different funnels. Left to right (i) *B. alba* under dummy funnel; (2) *B. alba* under *B. oleracea*; (3) *B. alba* under *B. alba*; (4) *B. oleracea* under dummy funnel; (5) *B. oleracea* under *B. alba*; (6) *B. oleracea* under *B. oleracea*.

Experiments With Growth-controlling Substances

I. The Reaction of Leafless Woody Cuttings to Treatment with Root-forming Substances

BY

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With Plates VII and VIII

INTRODUCTION

FROM quite early times (Sachs, 1882) it has been suggested that there may be specific substances present in plants which control root-formation. Loeb (1916, 1917) performed numerous experiments on the regeneration of Bryophyllum and observed that a stem segment bearing leaves will form roots more readily than one from which the leaves are removed. Van der Lek (1925, 1934) also found that the presence of leaves or buds promotes the formation of roots at the basal end of a cutting. The more recent work in the field of plant hormones has provided an elucidation of these results; it now seems very probable that the leaves and buds produce a root-forming substance which under suitable conditions travels to the stem and causes the initiation of roots. Thus F. W. Went (1929) found that a substance extracted from leaves and from germinating barley promoted root development in cuttings. The formation of roots by urine extracts applied to the internodes of a number of species has been observed by Laibach (1934) and Muller (1935). Laibach (1935) and Fischnich (1935) also caused the formation of adventitious roots on the stems of Coleus plants by applying pastes of 3-indole-acetic acid in lanolin. Went (1934) proposed the name rhizocaline for the root-forming substance. Thimann and Went (1934) showed that the active substance was present in large quantities in the auxin extracts obtained from Rhizopus and also from urine. Thimann and Went (1934) showed further that the pure auxins prepared by Kögl (1934*a* and *b*) and his associates were effective in root formation. Thimann and Koepfli (1935) found that 3-indole-acetic acid stimulates root production in pea cuttings. Hitchcock and Zimmerman (1936) have further shown that a number of synthetic substances including hetero-auxin will stimulate the production of roots on cuttings, two of the most active being indolebutyric acid and α -naphthalene-acetic acid, results which have been confirmed in this country and elsewhere, by Tincker (1936), Pearse and Garner (1937), and van der Lek and Krithje (1937), for cuttings of various species of plants.

It has been known for a long time that when woody cuttings are ringed adventitious roots will often form above the ring, and this would support the view that root-forming substances travelling downward in the phloem accumulate above the ring. Van der Wey (1932 and 1934) has shown that the transport of auxin in the *Avena* coleoptile is basipetal only, and Went (1933) that the transport of root-forming substances in *Acalypha* is polar. On the other hand, Hitchcock and Zimmerman (1935) found that several growth substances when applied to the soil in relatively high concentrations caused the formation of roots on the aerial stems of *Lycopersicum* and *Nicotiana*. The fact that concentrated solutions of root-forming substances are carried upwards in the transpiration stream in plants bearing rapidly transpiring leaves does not prove, however, that the normal or most-ready channel of transport is in the xylem. Cooper (1936), in a series of instructive experiments with hardwood lemon cuttings treated with hetero-auxin, concluded that there is some factor other than hetero-auxin concerned in root formation. He found that when applied to the base of cuttings, only strong solutions of hetero-auxin were effective in inducing root formation, and that cutting off the treated portion of the base eliminated the effect of the treatment, and further, that re-treating the remainder after cutting off the treated base caused no more roots to form than when not re-treated. To explain these results he suggested that a strong solution of hetero-auxin, when applied to the base, caused the rapid movement downwards of some substance already present in the leaves and stem, which is necessary for root formation. It has been suggested that the synthetic root-forming substances introduced by the workers at the Boyce Thompson Institute act in the same way. The object of the present work was to study the reaction of leafless woody cuttings to treatment with these synthetic root-forming substances. This paper gives some of the results obtained with leafless willow cuttings of *Salix vitellina* using indolebutyric acid; as this species readily roots from cuttings, and these would be expected to contain the root-forming substance. It was thought therefore to be a suitable material for use in determining if the effect of indolebutyric acid could be explained by its action on the mobilization of a naturally occurring root-forming substance.

MATERIAL AND METHODS

Indolebutyric acid, kindly supplied to the author by Dr. R. H. Manske of the National Research Laboratories, Ottawa, Canada, was used in all these experiments. The water solutions were prepared by dissolving the substance in the minimum amount of 95 per cent. alcohol and making up to the required volume with tap water. The lanolin preparations were made in the manner suggested by Laibach (1935). Leafless cuttings of *Salix vitellina* were used as the test subject. After treatment, the cuttings were set in fine silver sand in a propagating frame with bottom heat. The temperature of the rooting medium was not strictly controlled, but varied from 65° F. to 75° F. with the air temperature in the frames about 5° F. lower.

Experiment I. (October 1, 1937). The effect of applying indolebutyric acid to the base and to the apex of cuttings.

(a) *By lanolin method.* The cuttings were all approximately 4 in. long and were dormant at the time of treatment, so bore no leaves. There were four series, the first treated with lanolin only and the second, third, and fourth treated with lanolin containing 2, 6, and 10 mg. of indolebutyric acid respectively per gramme lanolin. In each series there were three groups, the cuttings in the first group being treated at the base only, the second at the base and apex, and the third at the apex only. Roughly 10 mg. of paste was applied in each case, and there were twenty cuttings in each treatment. Each cutting having treatment at the base and apex received therefore 20 mg. of paste while the others received 10 mg. The results obtained after the cuttings had been set in the rooting medium for one week are given in Table I.

TABLE I

Effect of Treating Dormant Willow Cuttings with Lanolin Paste containing Indolebutyric Acid. Time in Sand, 1 Week.

Treatment.	Conc. of indolebutyric acid. Mg. per gm. lanolin.	Average no. of roots per cutting.		
		Apical half.	Basal half.	Total.
Base	0	0.05	3.4	3.45
	2	0.1	6.3	6.4
	6	0.5	6.9	7.4
	10	0.3	6.4	6.7
Base and apex	0	0.1	4.1	4.2
	2	3.1	7.4	10.5
	6	6.0	9.1	15.1
	10	6.0	8.6	14.6
Apex	0	0.05	4.0	4.05
	2	4.6	7.4	12.0
	6	6.4	9.1	15.5
	10	6.05	8.2	14.25

The cuttings were arbitrarily divided into apical and basal halves for the purpose of recording.

Basal treatment therefore doubled the number of roots produced by the basal half of the cutting but had little effect on the number produced by the apical half. Apical treatment, on the other hand, enormously increased root production in the apical half as well as doubling that in the basal half. When both base and apex were treated the cuttings behaved much the same as when the apex alone was treated.

In the basal treated cuttings large swellings developed at the base and the bark often split, while the apically treated cuttings exhibited at the apex a similar swelling but to a less degree. Where both base and apex were treated the cuttings exhibited swellings at both ends, the basal ones being rather larger.

There was no clear relation between the number of roots formed and the

concentration of indolebutyric acid used, slightly more roots were initiated in each case however with the pastes containing the higher concentrations.

These results suggest that when the apex is treated the substance moves readily throughout the cuttings, but does not move upward to any extent when the base is treated. Further support for this suggestion is supplied by the fact that with apical treatment all the buds remain dormant, whereas with basal treatment the apical buds grew out in the same way as the cuttings in the control groups.

(b) *By solution method.* An experiment somewhat similar to that just described was carried out, using instead solutions of indolebutyric acid in water. The cuttings in this case were six inches long but otherwise similar. There were four series, the first treated with tap water only, and the second, third, and fourth treated with tap water containing respectively 8, 24, and 40 p.p.m. indolebutyric acid. There were two groups in each series; in the first the basal inch of the cuttings was immersed in the solutions, and in the second the cuttings were reversed and the apical inch immersed. The treatment time was for twenty-four hours, and after treatment the treated portions were rinsed in tap water, and the cuttings were then set upright in the rooting medium in the propagating frame. After a week in the sand they were removed, washed, and the roots counted, the cuttings again being divided arbitrarily into apical and basal halves for the purpose of recording. The results are shown in Table II.

The results obtained with treatment with water solutions agree with those obtained with the lanolin treatment in Table I. Thus the basal treatment has again doubled the number of roots formed from the basal halves of the cuttings without appreciably increasing the number formed from the apical halves, whereas apical treatment again enormously increased the number in the apical half as well as doubling that in the basal half.

Again, in the basal treated cuttings large swellings developed at the base, while the series treated at the apex exhibited similar swelling but to a less degree and only at the apex. The swellings were most pronounced in the highest concentrations, although again there was no clear relation between the concentration and the number of roots formed.

Photographs of some of the cuttings in this experiment are shown in Pl. VII, Figs. 1 and 2.

With the apical treatment all the buds remained dormant, whereas with the basal treatment the buds situated at the apex of the cuttings started to grow out in the same way as in the controls in each group.

Experiment 2. (October 11, 1937.) Effect of ringing.

There were three series of cuttings in this experiment, the first unringed, the second with a complete ring down to the wood in the middle of the cutting and the third with a three-quarter ring in the same position; after ringing the wound was coated with paraffin wax.

Each series consisted of a control group treated with tap water, and a group similarly treated with a water solution of indolebutyric acid at a concentration of 20 p.p.m. The treatment time was twenty-four hours, the apical inch of the cutting being immersed. After treatment the treated part of the cutting was rinsed in tap water, and the cuttings were then set in the propagating frame for two weeks before examination. Table III.

TABLE II

Effect of Treating Dormant Willow Cuttings with Water Solutions of Indolebutyric Acid. Time in Sand, 1 Week.

Treatment.	Conc. of indolebutyric acid solution. p.p.m.	Average no. of roots per cutting.		
		Apical half.	Basal half.	Total.
Base	0	1.4	6.4	7.8
	8	1.3	11.9	13.2
	24	2.6	13.4	16.0
	40	1.6	12.9	14.5
Apex	0	2.6	7.4	10.0
	8	13.9	9.0	22.9
	24	15.4	11.8	27.2
	40	13.8	13.6	27.4

TABLE III

Effect of Ringing on Root Formation of Dormant Willow Cuttings treated at the Apex with Water Solutions of Indolebutyric Acid. Time in Sand, 2 Weeks.

Treatment.	Conc. of indolebutyric acid. p.p.m.	Average no. of roots per cutting.		
		Above ring.	Below ring.	Total.
Unringed	0	0	1.9	1.9
	20	15.8	8.7	24.5
Complete ring	0	1.0	2.3	3.3
	20	14.5	2.8	17.3
Partial ring	0	0.5	3.5	4.0
	20	15.7	6.6	22.3

The figures in columns 2 and 3 of the unringed series refer to the apical and basal halves of the cuttings respectively.

With a complete ring the part of the cutting below the ring formed no more roots after treatment of the apex with indolebutyric acid than without treatment. With a partial ring the number of roots below the ring was increased twofold as compared with the complete ring. In the controls complete ringing had caused the formation of a few roots above the ring, and this was true also to a less degree with the partial ring.

Pl. VIII, Figs. 3 and 4, show that a complete ring caused, in the control group, the bud below the ring to grow out, and also in several of the cuttings treated with indolebutyric acid. With a partial ring, however, the bud below the ring remained dormant.

Experiment 3. (October 11, 1937.) The effect of removing the treated base, and re-treating the remainder at the new base.

There were two series of cuttings in this experiment; in the first the basal inch of the cuttings was treated, and then 2 in. cut off before planting, and the second series was similar but the new base was re-treated after cutting. Each series consisted of two groups, the first group being immersed in tap water for twenty-four hours and the second group treated for a similar time

TABLE IV

Effect of Cutting off the Treated Basal Portion, and Re-treating the Remainder at the New Base. Two Weeks in Sand.

Treatment.	Conc. of indolebutyric acid. p.p.m.	Average no. of roots per cutting.		
		Apical half.	Basal half.	Total.
Treated base cut off	0	0	1.9	1.9
" "	20	0	2.0	2.0
Treated base cut off and	0	0	2.3	2.3
new base re-treated	20	0	9.3	9.3

with tap water containing 20 p.p.m. indolebutyric acid. In the series which were re-treated the cuttings were allowed to dry partially and were then immersed for a further twenty-four hours after removal of the basal 2 in., in the hope that this would insure absorption of more of the solutions. The re-treatment was otherwise exactly similar to the original treatment; the cuttings in this experiment were 8 in. long, reduced after cutting to 6 in. After treatment the ends of the cuttings were rinsed as before and set in sand for two weeks before examination.

When the basal treated portions of willow cuttings are removed after treatment with indolebutyric acid no more roots are formed than on untreated cuttings. If, however, the cuttings are thus treated and then given a further treatment with indolebutyric acid solution the number of roots formed is again increased, showing that the cuttings are still capable of reacting to the substance (Pl. VIII, Fig. 5).

Experiment 4 (October 11, 1937). The position of the roots formed after treatment at the apex.

In this experiment forty cuttings were selected each having four nodes, and the apical portions of twenty of the cuttings were treated with a solution containing 20 p.p.m. indolebutyric acid, controls being similarly treated with tap water. After two weeks in sand the roots at each node and internode were counted. These figures are given in Table V, the nodes and internodes being numbered one to four from the apical to the basal end of the cuttings.

The number of roots formed at each node and internode became progressively less from the apex to the base after treating the apical end with indolebutyric acid solution, while the controls produced only a few roots at the extreme basal end.

TABLE V

The Position of the Roots formed after Treating the Apical Ends of Dormant Willow Cuttings with Solutions of Indolebutyric Acid. After 2 Weeks in Sand.

Average no. of roots per cutting.		
Node.	Apex treated with tap water.	Apex treated with indolebutyric acid solution.
1	0	6.2
2	0	5.0
3	0	3.0
4	1.9	2.5
Internode.		
1	0	2.2
2	0	1.3
3	0	0.8
4	0.2	0.4

DISCUSSION

Treating the basal ends of dormant willow cuttings with lanolin pastes or water solutions containing indolebutyric acid greatly stimulated the formation of roots at the basal ends of the cuttings, whilst if the apical ends were similarly treated increased root formation was evident throughout the cuttings and was not confined to the basal end. Cooper (1936) in his experiments with lemon cuttings concluded from his results after using 3-indole-acetic acid that some other factor than hetero-auxin was concerned in root formation. He put forward the theory that the treatment of the base of a cutting with a relatively strong solution of hetero-auxin caused the downward flow of some hypothetical root-forming substance already present in the cutting as an explanation of the increased root formation at the basal end after treatment. In support of this theory he claims that re-treating the base after cutting off the treated portion of lemon cuttings did not cause any further root formation. On the other hand, in these experiments re-treating the new base of dormant willow cuttings after removing the initially treated portion was still effective in causing renewed root formation. Further evidence that these cuttings were still reacting to the indolebutyric acid treatment was afforded by the swelling at the basal end after treatment. The explanation of this difference may lie in the method of treating the cuttings; in these experiments after the first treatment and removal of the basal two inches the cuttings were allowed to dry out partially before re-treating, in an attempt to insure that more solution would be taken up. Table V shows that after treatment of the apex the maximum response occurred at the most apical node, the number of roots formed gradually decreasing from the apex downward. While it is realized that the reaction of cuttings to treatment with indolebutyric acid is not a simple one, and that probably many factors contribute to the response; it is suggested that a logical explanation of these facts is that here indolebutyric acid itself was the active agent concerned in promoting root formation, and was

itself used up or chemically changed in the process. The decreasing number of roots at each node from the apex when the apical end was treated would thus be explained by a concentration gradient of indolebutyric acid within the cutting. There seems from these results no need to postulate the redistribution of a root-forming substance by the indolebutyric acid as it is clear that any portion of the cutting was capable of reacting to the acid. The fact that the apical treatment with indolebutyric acid kept all the buds on the cuttings dormant also supports the view that it was itself the active agent, and did not act through the movement of some substance already present in the cuttings.

The results from the ringing experiments show that with a complete ring the portion of the cuttings below the ring formed no more roots after treatment than when not treated; with a partial ring, however, more roots were initiated in the portion below the ring after treatment than in the controls in this group; while on the portion above the ring treatment caused just as many roots to form as with a complete ring. It would seem to be clear from these results that after treating the apex of cuttings with indolebutyric acid, it is transported downward in some region of the stem external to the xylem. Furthermore, the fact that while the number of roots produced in the portion of the cuttings above the position of the ring in the unringed, the partially ringed, and the completely ringed series was practically the same after treatment, the number of roots initiated in the portion below the position of the ring was in descending order in these three series, would seem to indicate that up to a certain point the level of indolebutyric acid in any region of the stem was a limiting factor for root formation.

In cuttings of *Salix vitellina* the normal root formation is mainly nodal, and while after treatment the number of internodal roots formed was increased the major effect was greatly to strengthen the nodal root formation. This may be due to a tendency for the natural hormone to accumulate at the nodes in untreated cuttings, and a similar tendency for indolebutyric acid also to accumulate at the nodes in treated cuttings, or it may be due to the distribution of the dormant root initials already present in the cuttings. The results from experiment I indicate that beyond a certain point increasing the concentration of indolebutyric acid did not increase the number of roots formed, and it is probable that at this point all the dormant root initials had grown out, and some other factor limited root formation; this factor may possibly have been the amount of stored reserves present in the cuttings.

These results indicate that it may be more effective to treat dormant woody cuttings at the apical end when applying root-forming substances. The dormancy of the buds thus induced presents a difficulty since during their period of dormancy the roots will be growing at the expense of the stored materials in the cuttings. How long this dormancy persists is a point that will be investigated, but in any case it may be possible to overcome the difficulty in question by supplying the solutions through cuts below the buds towards the apex of the cuttings.

SUMMARY

1. Treating the basal ends of dormant willow cuttings with lanolin pastes or water solutions containing indolebutyric acid greatly stimulated the formation of roots at the basal ends of the cuttings.
2. When the apical ends were similarly treated root formation was accelerated throughout the length of the cutting and was not confined to the basal portion.
3. Ringing experiments indicated that indolebutyric acid was transported downward in the region of the stem external to the xylem.
4. After treatment of the apical portion of cuttings the maximum response occurred at the most apical node, the number of roots formed gradually decreasing from the apex to the base.
5. When the portion of the base of the cuttings which had been treated was removed, the effect of the treatment was eliminated.
6. When the treated portion of the base was cut off a further treatment with indolebutyric acid again caused a response.
7. These results suggest that in these experiments indolebutyric acid itself was the active agent concerned in promoting root formation, and was itself used up or chemically changed in the process.

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EXPLANATION OF PLATES VII and VIII

Illustrating Dr. H. L. Pearse's paper on 'Experiments with Growth-controlling Substances. I. The Reaction of Leafless Woody Cuttings to Treatment with Root-forming Substances'.

PLATE VII

Fig. 1. Cuttings of *Salix vitellina* treated at base with water solutions of indolebutyric acid. From above downwards, control tap water, 40 p.p.m., 24 p.p.m. and 8 p.p.m. for twenty-four hours respectively. After 1 week in sand.

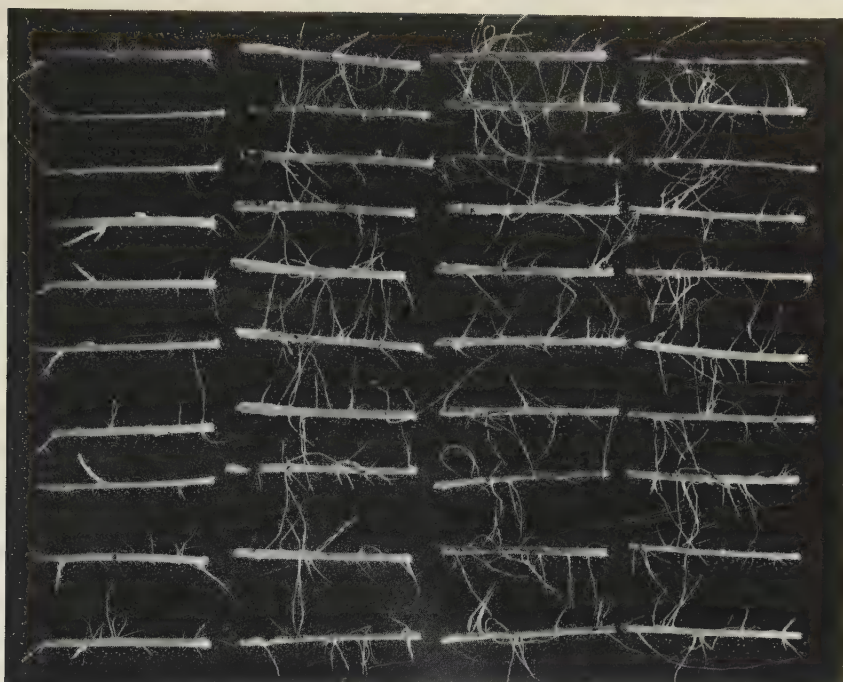
Fig. 2. Cuttings of *Salix vitellina* treated at apex with water solutions of indolebutyric acid. From above downwards, control tap water, 40 p.p.m., 24 p.p.m., 8 p.p.m. for twenty-four hours respectively. After 1 week in sand.

PLATE VIII

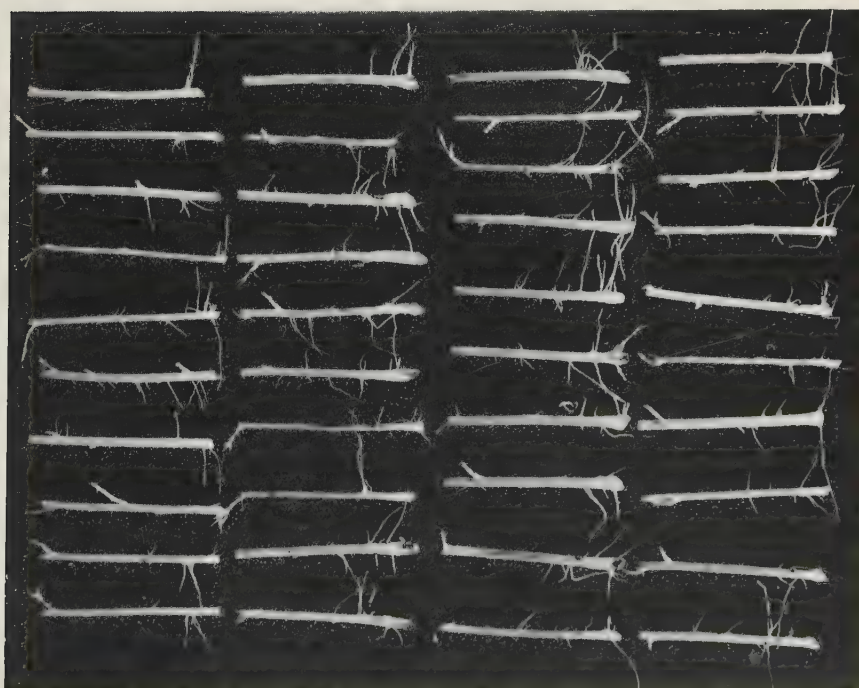
Fig. 3. Cuttings of *Salix vitellina* completely ringed down to the xylem in the middle, and treated at the apex for 24 hours. Upper row, tap water; lower row, tap water containing 20 p.p.m. indolebutyric acid. After 2 weeks in sand.

Fig. 4. Cuttings of *Salix vitellina* three-fourths ringed and treated at the apex for twenty-four hours. Upper row, tap water; lower row, tap water containing 20 p.p.m. indolebutyric acid. After 2 weeks in sand.

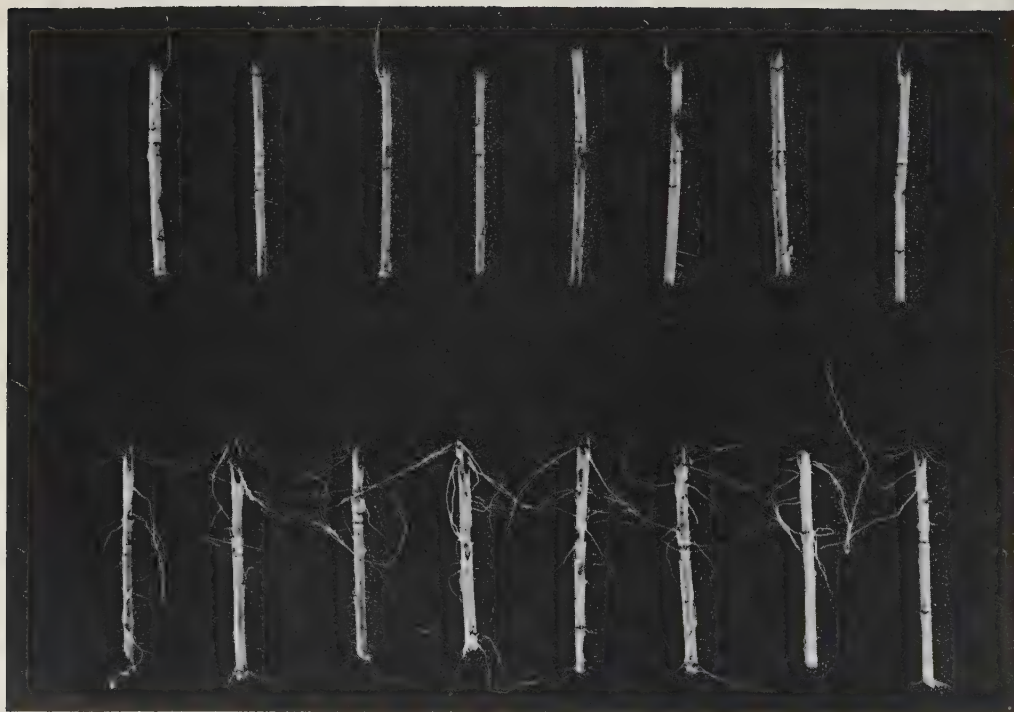
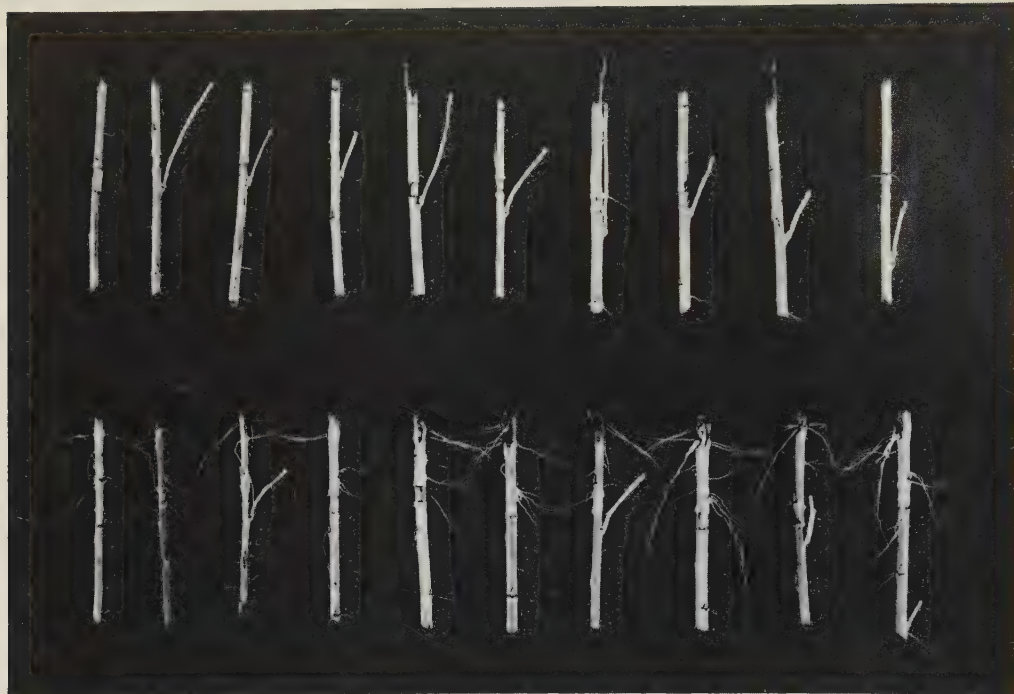
Fig. 5. Cuttings of *Salix vitellina*. Effect of re-treatment of new base after cutting off the previously treated basal portion. Upper row, treated at base of stem with indolebutyric acid (20 p.p.m.) for twenty-four hours and 2 in. removed before planting. Lower row, a similar series but re-immersed in solution for a further twenty-four hours after the treatment just described. After 2 weeks in sand.



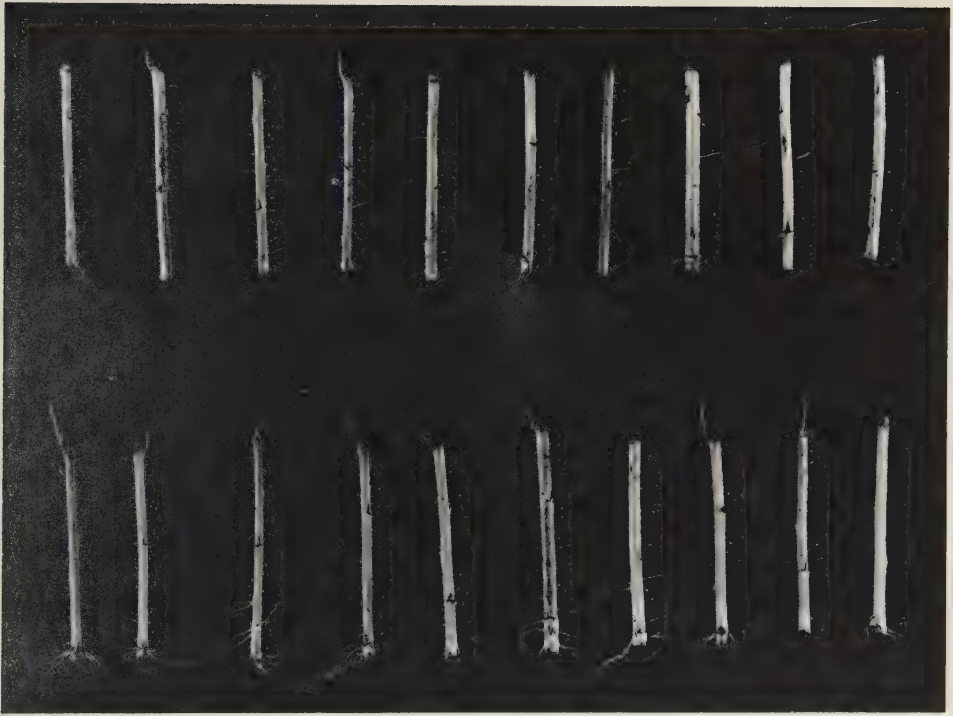
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PEARSE — WOODY CUTTINGS TREATED WITH ROOT-FORMING SUBSTANCES.



Studies in Vernalisation of Cereals

II. The Vernalisation of Excised Mature Embryos, and of Developing Ears

BY

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With Plate IX and two Figures in the Text

THE process of vernalisation by low temperature is in practice carried out on the germinating grain. Any attempt to analyse the causal factors concerned in the process must necessarily take account of the complex nature of the mature fruit of the cereal plant, and must inquire into the possible part played by changes occurring in the various tissues present. In this connexion the following parts are likely to be of importance: (1) the embryo proper, (2) the endosperm, (3) the aleurone layer, and (4) the integuments. The important part played by the endosperm in the nutrition of the embryo is well known. The details of the process in the barley plant which were very thoroughly studied by Brown and Morris (1890) appear to be similar in all cereals, and nothing essentially new has been added by later investigators.

Attempts to account for the vernalising effect of low temperature on the basis of the changed nutrition of the embryo or changed metabolism of the endosperm have been made by several Russian investigators. Thus the changes in carbohydrate and the associated enzyme activity during the process of vernalisation were investigated by Demkovskii (1932), while the behaviour of nitrogenous substances was studied by Konovolov and Rogalev (1937). Richter, Rancan, and Pekker (1933) established changes in the enzyme concentrations and in the iso-electric point under vernalising conditions. Again, indirect methods have been used by Krasnoseliskaia-Maximova (1931), in which portions of the endosperms of spring and winter varieties, and of vernalised and unvernalsed grains were interchanged. She showed that the inserted fragments influenced the flowering behaviour of the grains receiving them, and concluded that the active factor was of a hormonal nature. Serieskii and Sluckaia (1934) carried out similar experiments, but did not obtain positive results. In view of the fact later to be established that the vernalising process can be carried out on the embryo, completely separated from the endosperm, it is not necessary to discuss these results farther, as

evidently, in spite of their intrinsic interest, the facts recorded cannot be used as an explanation of vernalisation.

In view of a possible hormone control of the onset of flowering, the evidence for the presence of specific hormones in the grain is clearly important. Cholodny (1935) has demonstrated the presence in large quantities in the endosperm of a hormone resembling auxine in many of its physiological effects, which, however, differed in the details of its action sufficiently to be given a separate name. This so-called 'Blastenin' has been used as a basis of an explanation of vernalisation by Cholodny (1936), and his theory will be discussed in due course. Suffice it to say here that this hormone is confined to the endosperm, in which it appears only after water absorption, and is then present even in the dead tissue. It is readily absorbed by the embryo, and is, according to its discoverer, particularly associated with the process of starch hydrolysis. The integuments and aleurone layer are impermeable to this hormone, which is thus confined within the embryo sac.

A second hormone system has been isolated from the grain of maize by Dagys (1937) which, apparently, is identical with the 'Bios' required for the cell division of yeast. This hormone in the resting seed is confined almost exclusively to the aleurone layer, and is transferred to the developing embryo so that five- or six-day-old seedlings contain much of it in the leaves, coleoptile, and root. Particularly interesting is the fact that the hormone is set free during proteolysis.

These results of Dagys throw light on the essential nature of the aleurone layer in the germination of rice and other grasses as studied by Schander (1934). By removing the aleurone layer, or merely by severing the connexion between this layer and the embryo by 'ringing' the scutellum, germination of the grain is prevented. Schander concluded that the stimulus for germination depends on the translocation to the embryo of an activating substance, which occurs during the first few hours of germination. Six hours is enough, in the case of rice, to eliminate the effect of breaking contact between the scutellum and the aleurone layer. It should, however, be noted here that Brown and Morris (1890) succeeded in growing embryos excised from dry grains of barley, and this has also been achieved in the case of rye in this laboratory. The importance of Schander's investigation in relation to the work here reported concerns the time of removal of the embryo, and the possible transfer of activating substances before that time.

The possibility of growing excised embryos when supplied with nutrient salts and available carbohydrate has been known for a long time. Andronescu (1919) grew maize embryos on different nutritive solutions, and obtained best results with 15 per cent. sucrose. Esenbeck and Suessenguth (1925) found that excised maize embryos grew well on sterile starch agar. Laibach (1929) reports that immature embryos of *Lolium* hybrids attained the condition of those in ripe seed when removed from the seed and grown in 15 per cent. sucrose. Brown and Morris (1890) also studied the nutrition of such excised

embryos in barley, and showed that sucrose is particularly favourable for their growth. In the absence of added carbohydrate growth soon ceases but is renewed when sugar is given. Schander apparently added no carbohydrate, and thus obtained only a limited amount of growth.

It appeared important to test directly the possibility of vernalising the excised embryo and thus conveniently to separate the effects of low temperature on the endosperm from its effect on the embryo itself. This technique has proved completely successful, and results have already been briefly reported by the authors (Gregory and Purvis 1936a).

The vernalisation of excised embryos.

In 1935 embryos of winter rye (variety Petkus) removed from the endosperm were found to germinate and grow readily on nutrient agar as used by Robbins (1922) for the culture of isolated root tips. The grain was sterilized by soaking for five hours in a solution of calcium hypochlorite containing 1 per cent. chlorine as used by Wilson (1920). The correct exposure and concentration were determined by trial. A preliminary rinse of the dry grain in 70 per cent. alcohol for a few seconds facilitated thorough wetting of the material by the sterilizing solution. The embryos were easily removed with a flamed spear-headed needle, and carefully transferred to plates of nutrient agar. A number of embryos were kept at a temperature of 1° C. until germination was well established. After sixteen days a further batch was excised and germinated at room temperature. The resulting seedlings were transferred to sand cultures when the vernalised embryos had received three weeks treatment. Eventually some of the treated plants came into ear, while others were no more advanced than the controls. As three weeks is far short of the optimal vernalisation period for winter rye the experiment was repeated in 1936.

About fifty excised embryos were kept at 1° C. for six weeks, and five days before the end of this period the control embryos were excised and germinated at 18° C. The sturdiest seedlings from both lots were planted in soil. To check the effect of a free supply of glucose in the culture medium during germination a further lot of whole grains was sterilized and germinated on the same nutrient agar at both temperatures.

The results of this experiment are shown in Table I.

It is evident that the isolated embryos react in the same way to low temperature as do the whole grains, and to the same degree. In all, eighteen vernalised embryos were grown to full maturity, and every one of these had set seed before any single unvernalsed plant reached the stage of shooting. The characteristic reduction in leaf number appears in the vernalised plants and, moreover, the plants from excised embryos show only one less leaf than those from vernalised whole seeds. This apparent difference may possibly be attributed to damage of the embryo and failure of the first leaf. A reduction in tillering was also noted in the embryos and whole grains germinated

at 1° C. Normal ears were found in plants grown from the excised embryo, although some of the plants resulting were smaller than the controls grown from whole seed. Treated and control plants grown from excised embryos are shown in Pl. IX, Fig. 1.

TABLE I
Vernalisation of Excised Embryos and of Whole Seeds of Winter Rye
(*var. Petkus*).

Experimental material used.	Excised embryos.		Whole grain.		Whole grain.	
Medium of germination.	Glucose agar.		Glucose agar.		Sand.	
Temperature of germination.	1° C.	18° C.	1° C.	18° C.	1° C.	18° C.
Final leaf no.	9.4	> 21	10.5	> 21	11.5	> 21
Days to first anthesis	74	> 150	70	> 150	68	> 150
Mean time to anthesis (days)	89.3	> 150	81.2	> 150	68.5	> 150

The vernalisation of developing embryos.

The fact that the vernalisation process occurred in the embryo apart from the endosperm suggested the possibility of applying the low-temperature treatment after anthesis, during the period of development of the embryo and before dormancy set in.¹ During 1935 a preliminary experiment was performed. Chilling of the ripening ears was carried out in two ways: (1) The ears together with several nodes of the stem were cut off and kept in water in a refrigerator for 5 weeks at 1° C. Control ears similarly treated were kept in a dark room at normal temperature until the grain ripened off. After the low-temperature exposure the treated ears were allowed to complete ripening at room temperature. (2) The second method consisted in treating ears attached to the plant. The selected ears after anthesis were inserted into wide glass test-tubes, and kept in place by plugging with cotton-wool. In one set these tubes were placed in the necks of vacuum flasks containing crushed ice. The ears were thus kept at low temperature but did not come into contact with free water. Control ears were similarly placed in flasks without ice. The arrangement is shown in Pl. IX, Fig. 2. In all, the ears were thus treated for twenty-four days and were then allowed to ripen normally in air. The seed thus obtained was sown the following year on March 17, 1936, in pots of sand, without further low-temperature treatment.

Flowering of these plants was irregular, especially in those treated in vacuum flasks, where the time of treatment was less than the known optimal exposure. With both methods of chilling however, flowering was appreciably hastened, as may be seen from the figures in Table II. This result has already been briefly described (Gregory and Purvis 1936*b*) and was sufficiently encouraging to warrant repetition in the next year, when longer periods of treatment were used.

¹ Kostjucenko and Zarubailo (1936) record a natural vernalisation in the ear during ripening. A review of the work of these authors appeared in *Herbage Reviews* (1937). The effect of temperature during ripening of seeds of wheat on subsequent flowering behaviour is clearly demonstrated. No direct experiments on chilling the ear were performed.

In 1936 a larger number of ears was treated. The method used was that of placing ears attached to the stem in a refrigerator, and also, in some cases, whole plants in pots were so treated. The period of chilling was forty-five days. Final ripening was carried out at room temperature. The ears were air dried and the grain sown in sand culture on May 31, 1937, without further treatment. The results of this experiment are shown in Table III and in Pl. IX, Fig. 3.

TABLE II

Effect of Vernalising Developing Ears of Winter Rye (var. Petkus), 1935.

Method of treatment.	Time of treatment.	Days from planting to anthesis in resulting plants.	
		Ripened at 1° C.	Ripened at normal air temperature.
Cut ears in water	5 weeks	102	146
Attached ears in vacuum flasks	24 days	110	164

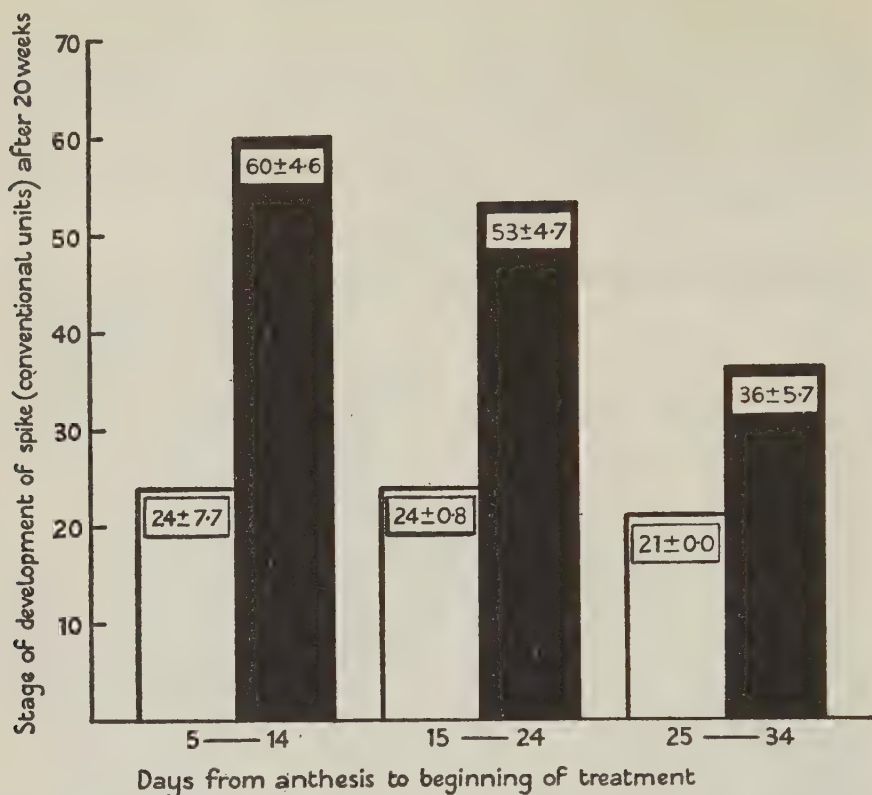
TABLE III

Vernalisation of Growing Embryos in the Developing Ear of Winter Rye (var. Petkus), 1936.

Treatment.	No. of plants.	Condition of plants 15 weeks after planting.		
		Stage of flower initiation.	Stage of fully formed ears.	Maturity in conventional units.
Vernalised	156	65%	38%	53
Control	49	49%	0%	24

The results leave no doubt as to the efficacy of vernalisation during ear formation, although it appears that not all the treated grains had attained the vernalised state. Thus only 38 per cent. of the treated seeds produced plants with fully emerged ears, though others were approaching this stage, whereas, of the control no single plant produced a fully formed ear. The last column in the table requires some explanation. Here the stage of maturity reached is stated in conventional units, which were derived from the approximate durations (in days) of the successive stages in development in fully vernalised plants. The actual values are shown on p. 242. At the time of examination each plant was dissected and the stage reached by the meristem of the main axis determined, and assigned a 'score' on the basis given. The mean scores are entered in the last column of Table III.

A further point investigated in this experiment was the relationship between effectiveness of chilling and the age of the embryo when treatment began. At anthesis ears were dated, and the age of the treated embryos was thus determined. The results of the experiment could therefore be grouped into ten-day classes covering the period from five days to thirty-five days after anthesis. The results are presented graphically in Text-fig. 1. It is apparent that the treatment is effective from the earliest stage of the



TEXT-FIG. 1. Relation between age of embryo at beginning of vernalisation in the ear, and effectiveness of the treatment. Black=vernalised; white=unvernalised controls.

For details see text.

Conventional Units for Evaluating Stage of Development Reached by Spike.

	Units.
Simple ridges—very short spike	10
" long spike	15
Double ridges	21
Ridges swelling	24
Lateral initials branching	26
Flower initials appear	28
Stamen " "	30
Stamen lobes appear	33
Awns growing	35
Spike elongating, stamens covered by palea	37
Spike emerged from last leaf	39
Anthesis	49
Past anthesis	49 + number of days from anthesis to time of examination.

embryo, and decreases in its action as the time of beginning the treatment is delayed.

This is no doubt due to the circumstance that the low temperature is effective only during the period of active growth of the embryo and ceases as the embryo becomes dormant. Clearly therefore this is a question of duration of the exposure to low temperature which has been previously described (Purvis and Gregory, 1937). The control and experimental seeds in the present experiment were obtained from ears cut off with the stem attached and placed in water at the same time, and in each set ears of similar dates of anthesis were included. The experimental plants alone were kept in the refrigerator. The agreement between the control values shows that there is little or no difference in behaviour among the progeny of ears removed at various times after anthesis and ripened in the manner described. The wide range of anthesis dates among the parent plants made possible a comparison between 'earliness' of parent and offspring: no indication of inheritance of this characteristic was found.

Immature ears.

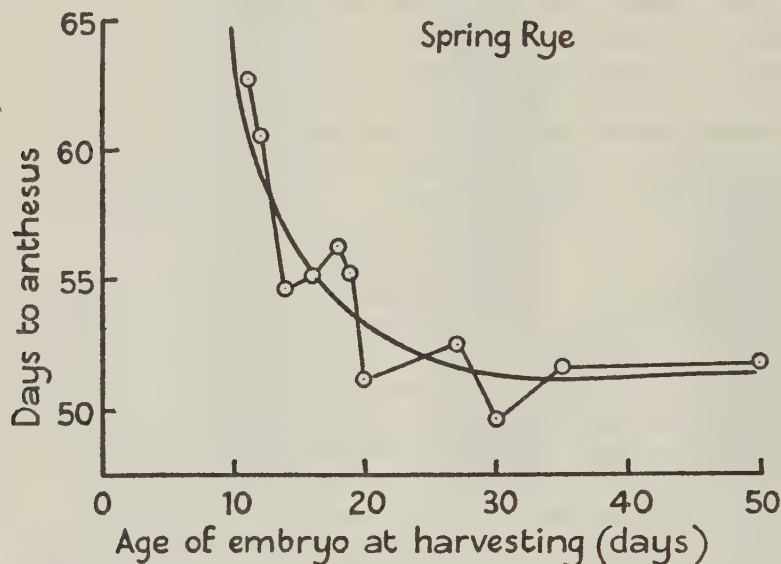
A second series of controls for the experiment described above was afforded by seeds from ears which were removed with their stems from the parent plants at varying known times after anthesis; these were immediately air dried. Thus seed was obtained from ears removed from the plant as early as five days after anthesis. Quite unexpectedly these seeds germinated on sowing in the following spring, and yielded completely normal plants, although the individual grains were very small (4×1 mm.) and apparently without reserves. The germination of such immature seeds in barley had, however, been recorded by Harlan and Pope (1922), and Harlan (1926). The detailed structure of these viable immature fruits of rye is now being investigated in this laboratory. In the case of winter rye the plants grown from seeds of such a range of maturity (5–50 days) showed no differences in the developmental stage reached after seventeen weeks. Since the plants were unvernalsised no ears emerged.

A similar series of immature grains of spring rye, however, showed a variation in anthesis date from 51.8 ± 1.17 days (mean of 10 plants) in completely matured seeds to 62.7 ± 0.91 days (mean of 3 plants) in grain from ears removed eleven days after anthesis, the earliest ears to give viable grain. The results for spring rye are shown graphically in Text-fig. 2, in which progressive decrease in time to flowering is shown with progressive maturity of the grain. There is some suggestion here that very immature grains of spring rye are *partially devernalsised*, and more information on this point will be obtained.

The experiments described above were also carried out on four varieties of wheat supplied to the authors at the request of Professor Maximov.¹ Unfortunately no success in ear vernalisation was achieved with any of these

¹ The varieties used were *Lutescens* 329 and 1060/10, *Hostianum* 237, and *Erythrospermum* 'Kooperatorka', the first two very hardy, the second pair less so. We wish to thank Dr. Nina Meister of Saratov for sending us these seeds.

varieties although vernalisation at germination was successfully carried out with all varieties, the effect of six weeks' treatment on *Lutescens 329*, however, being very slight.¹ On the contrary, grains from immature ears of wheat removed from the plants five days or more after anthesis were successfully grown, but it was found, as in winter rye, that no relationship existed between the age of the embryo on removing the ear and the stage of development attained by the resulting plant in seventeen weeks.



TEXT-FIG. 2. Spring rye. Relation between age of embryo at harvesting and time to anthesis in resulting plants.

The effect of drying out vernalised seeds.

The failure of these experiments with wheat raises an interesting problem. It is possible of course that failure was due to faulty technique, although exactly the same method was used as proved adequate for rye. It may, on the other hand, be associated with the fact that the embryo of wheat is much smaller than that of rye, and arrest of growth and onset of dormancy may occur so soon that the period of vernalisation is too short to exert a recognizable influence on the time of ear emergence.

The whole question of the relation of water content to the vernalisation process remains somewhat obscure. In the experience of the authors the Russian technique of vernalisation, which depends on restricting the water supply of the grain, is difficult to carry out, and at the level of water content recommended (50 per cent. of dry weight) the least drying out entirely in-

¹ Failure in the case of 'Kooperatoroka' and 'Lutescens 329' is interesting as Kostjucenko and Zarubailo used these varieties with success.

hibits the process as Lojkin (1936) also found. For this reason, chilling has generally been carried out in moist sand in the present work. Lojkin shows that wheat (Turkey Red) maintained during chilling at 50 per cent. water content will later 'head out', but if the water content is allowed to fall, no vernalisation at all results. It appears that even when the initial level of moisture content is 55 per cent., a gradual reduction leads to failure of vernalisation, and with an initial content of 60 per cent. and gradual reduction of this percentage only 12 per cent. of the plants are vernalised. It would appear therefore that the vernalisation achieved in the earlier stages of germination is later completely annulled by drying.

Experiments on drying out vernalised seeds of wheat (var. Turkey Red and var. Ukrainka) have been recorded by Lojkin (1936) and Lebedev and Sergejev (1936). The former investigator found that completely vernalised seed kept air dried for four weeks at 1° C. and 15° C. showed either partial or complete loss of vernalisation. The Russian investigators found a similar situation to hold, and in both cases variable loss of germinative capacity is recorded. Lebedev and Sergejev account for the result by suggesting that the growing-point of the main axis is injured during drying, resulting in stimulation of tiller production, such tillers being unvernalsed. Our experience is contrary to both these contentions. Examination of seedlings from vernalised winter rye kept for ten weeks after re-drying showed no visible injury to the growing-point, and germination proceeded in a normal manner, but the plants were found to have lost the capacity for early ear emergence. Again, in general, the tillers of vernalised plants behave in exactly the same way as the main axis and, further, an experiment on this point was performed in 1934. In this experiment the main axis of vernalised winter rye plants was deliberately removed as soon as possible after germination, and in turn the tillers were also removed as they appeared over a period of some weeks. Finally, a few tillers of high order were allowed to develop, and produced ears at a much earlier date than did the main axes of unvernalsed and unmutated plants. The vernalised state was thus transmitted through some generations of tillers. This experiment was purely qualitative, but quite conclusive. The data obtained on the effect of drying out vernalised winter rye in 1936 are presented in Table IV.

The grain in every case was vernalised according to the Russian technique for a period of six weeks, and then exposed to laboratory air for forty-eight hours, and subsequently stored in corked tubes. Vernalisation was started at such different dates that all variants could be sown simultaneously. From the data in the table the following conclusions may be drawn. (1) Vegetative growth as shown by the tiller production is greatly increased by re-drying vernalised seed, and this effect increases markedly with the time elapsing before sowing. (2) The tendency to flower as measured both by length of spike and by the conventional 'scoring' method (described on p. 242) decreases rapidly as the period over which the seed is kept dry is prolonged beyond six weeks. (3) As far as tendency to flower is concerned the seeds dried for twenty

weeks are identical with the unvernalsed controls. Apparently therefore the effect on flowering of the previous vernalisation has been completely removed.

TABLE IV

Effect of Drying Vernalised Grain for Varying Periods on After Development

	Period of drying.						Unvernalsed, sown dry.
	1 day.	6 weeks.	8 weeks.	12 weeks.	14 weeks.	20 weeks.	
Tiller number per plant (10 plants per pot) .	2.7	2.9	9.7	10.9	16.3	13.7	4.7
Spike length in mm. .	34.0	44.3	4.9	2.8	2.4	1.6	1.6
Stage of development: 'score' after 19 weeks' growth	51	49	26	25	21	20	19

DISCUSSION

The experimental results with excised embryos suggest that the hormone 'blastenin' demonstrated by Cholodny (1935) to exist in large quantities in the endosperm is not immediately concerned in the vernalisation process. Very briefly stated, Cholodny's theory is that vernalisation essentially consists in a shortening of the life cycle by hastening each cell in turn through its various stages of development. Such an acceleration was shown in the case of the root-apex cells of maize by Cholodny (1936), and on the basis of this finding he extended the hypothesis to cover the complete development of the plant and so account for earlier flower formation in vernalised plants. The experiments of one of the present authors (Purvis, 1934) have shown, however, that up to the stage of flower initiation developmental processes go on at the same rate in vernalised and unvernalsed plants; thus, the leaf production rate is the same, and there is no indication of earlier senescence in individual leaves of vernalised plants. Tiller production, moreover, proceeds more rapidly in the unvernalsed plants. Cholodny's theory, therefore, appears to be inadequate.

The question remains whether hormone production is at all affected by vernalisation. In this connexion the 'bios' type of hormone, isolated by Dagys (1937) would appear to demand attention. Schander (1934) has claimed that even a few hours' soaking in water suffice to mobilize from the aleurone layer an activating substance which one may assume to be the 'bios' found by Dagys. In the experiments here described the seeds were soaked for five hours in a sterilizing solution, during which time such a transfer may have taken place. It must, however, be stressed that this soaking took place at room temperature whether the embryos were afterwards vernalised or not, and moreover, was carried out on grains that were to be germinated whole as well as on those from which embryos were later excised. This transfer therefore cannot be the cause of the differences between the excised embryos,

chilled and unchilled, but the proximal cause must then be a differential effect of low temperature on the later action of the hormone thus transferred.

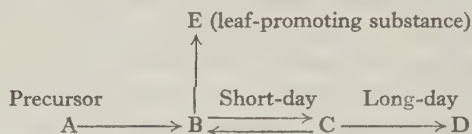
In the previous paper (Purvis and Gregory, 1937), in which the effect of duration of low temperature treatment was described, it was shown that the effect of four days' treatment was barely demonstrable, while more than three months were required to convert a winter rye into a state indistinguishable, so far as flowering is concerned, from spring rye. Comparing also the effects of vernalisation of excised embryos in 1935 and 1936, the extended period of six weeks used in 1936 had a greater effect than three weeks in 1935. If therefore this effect of duration of treatment acts by virtue of an accumulation of hormone this occurs alike in the excised and in the whole grain, and therefore cannot be due to a gradual transfer from the aleurone layer throughout the period of treatment. The effect noted by Schander can thus only be of importance in this connexion if (1) the hormone transferred is autocatalytic in its action, and (2) this autocatalysis takes place at a greater rate at low temperatures than at high, thus leading to a greater accumulation in the chilled embryos. Such an autocatalytic accumulation was, in fact, suggested in the previous paper (Purvis and Gregory, 1937) to account for the gradation of vernalisation by varying periods of low temperature and of short days.

Returning for a moment to the 'blastenin' of Cholodny, which is associated with the hydrolysis of starch, if indeed this resembles auxine in its action and is essential for extension growth, its presence in the developing embryo must be postulated. Yet, Brown and Morris (1890) showed very clearly in the case of barley that the starch of the endosperm is not attacked until the cell walls of the endosperm are dissolved by a cytase, and this does not take place until the radicle emerges twenty-four to thirty-six hours after germination begins. The five hours' soaking is not sufficient to allow of blastenin transference, and clearly therefore the embryo has the power of synthesizing this or a similar hormone from glucose and inorganic salts. It may perhaps be legitimate to assume that the 'bios' hormone can similarly be synthesized, in which case there is no obligate dependence of the embryo on the seed reserves for growth, and the vernalisation process is thus also independent of the hormone equipment of the grain.

The demonstration of vernalisation during embryo development throw further light on this question. Reference may here be made to a speculation of Ljubimenko (1933) in relation to photoperiodic induction which, as has been shown (Purvis and Gregory 1937), results in the case of winter cereals in 'short-day' vernalisation. He suggests that 'if the process of induction were started from the moment at which the zygote came into existence by fertilisation . . . there seems no reason why intervening somatic cells should not be almost indefinitely curtailed so that the zygote might develop almost directly into reproductive tissue. . . .' (quoted from I. A. B. Bull. 17, p. 19). The experiments on ear vernalisation of winter rye show that this is not the case with low temperature vernalisation. These experiments further show that the process of vernalisation had begun soon after the embryo had started

development, i.e. from five to fifteen days after anthesis. At the earliest stage (6 days) approximately eight cells are present in the embryo and the endosperm at this time is represented by free nuclei and contains no accumulated starch (unpublished data). Clearly therefore the presence of starch which Chlodny postulated as requisite for the formation of blastenin is not essential for the action of low temperature; neither at this stage is there any aleurone layer present, although the peripheral nuclei in the embryo-sac later give rise to this tissue. The embryo at this stage therefore resembles more closely in its nutrition the excised embryos dealt with above. It is growing rapidly, and must be absorbing materials all over its surface directly from the contents of the embryo sac, in the form of soluble carbohydrates and probably soluble nitrogen compounds. Early in development also the embryo may be removed from the embryo sac and grown on nutrient media as used for mature excised embryos. Clearly, therefore, the embryo in a comparatively early stage can also synthesize growth hormones from glucose, inorganic nitrogen and salts. Again, in so far as flowering is controlled by a specific hormone in the developing embryo, as in the mature embryo at germination, the accumulation of such hormones takes place more rapidly at low temperatures, as the data in Tables II and III show. It is true that a similar experiment with wheat failed to show a vernalising effect during development of the embryo, but this may be due to occurrence of dormancy of the embryo at so early a date that the time available for low temperature to act is too short.

As shown in Table IV, the mature embryo after having been vernalised can be again devernalsed by maintaining it in a dry condition for twenty weeks, and vernalisation thus appears to be a reversible process. This reversal is slow, and is not apparent at all until after six weeks' dry storage. In the previous paper (Purvis and Gregory, 1937) it was shown that vernalised winter rye can be kept in a moist condition at 1° C. for six months without reversal taking place, whereas it now appears that twenty weeks of dry storage at room temperature is sufficient to annul the effect of vernalisation in some respects. Referring to Table IV, it will be seen that in so far as the stage of development in spike length or in the conventional 'score' units are concerned, complete devernalsation has occurred, but in the effect on tiller number an after-effect of the previous low temperature is apparent. This effect seems to be in agreement with the schema of vernalisation already suggested (Purvis and Gregory, 1937). This schema is here reproduced:



There it was assumed that by autocatalysis from a precursor (A) a substance was produced which accumulated more rapidly at low temperature. This substance (B) could either produce a 'flower-initiating' (C) or a vegetative

'leaf-forming' substance (E) according to the conditions prevailing (day length or temperature) and this system $C \rightarrow B$ is reversible. It would appear that the devernalisation due to drying also leads to a reversal of the system; the accumulated precursor of the flower forming substance (B) which in the imbibed grain remains as such, is in re-dried grain at room temperature converted into the 'leaf-forming vegetative substance (E). Thus the original high content of B is reflected in the dried-down grain as an increase in vegetative vigour and very high tiller number (14 weeks drying, 16.3 tillers; unvernalsed, 4.7 tillers). Lebedev and Sergejev (1936) as stated on p. 245 also record greatly increased tillering in dried-down vernalised grain. Thus in winter wheat (Kooperatoroka) unvernalsed plants produced from four to twenty tillers, while those which had been vernalised and then stored dry for a complete year produced from 73 to 145.

There appears to be a difficulty here, since if the drying down of the vernalised mature embryo leads to disappearance of vernalisation, it might be expected that the embryos exposed to low temperature during development would later lose this effect while dormant. Manifestly this is not so in the case of winter rye, though the failure of ear vernalisation in wheat might be interpreted in this way. At present there is no clear solution of these apparently contradictory results. Suffice it to say that the onset of dormancy of the embryo is not in any sense a process of mere passive drying. At this stage the embryo is completely drought resistant, which is a unique condition in the life of the plant, and involves many unknown factors.

The complete success attending experiments on the vernalisation of excised embryos indicates clearly that low temperature acts directly on the embryo itself and is not due to an indirect influence on the endosperm of aleurone layer afterwards transmitted to the grain.

SUMMARY

1. Embryos of winter rye (var. Petkus) excised from the grain and germinated on agar containing glucose and mineral nutrients have been successfully vernalised by low temperature.

2. Low-temperature vernalisation has also been carried out on ripening grain while still in the ear.

3. From these experiments it is concluded that the process of vernalisation is localized in the embryo itself and is entirely independent of changes in the endosperm or aleurone layer which may take place during germination.

4. It is concluded therefore that the growing embryo is able to synthesize hormones from a simple substratum containing glucose and inorganic salts (including nitrates).

5. The loss of vernalisation by drying down the vernalised grain for periods longer than six weeks has been demonstrated by experiment. Although the effect on flowering vanishes, an after effect is shown by the high tiller production; this is attributed to a reversal in hormone synthesis.

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EXPLANATION OF PLATE IX

Illustrating Prof. F. G. Gregory's and Dr. O. N. Purvis's paper on 'Studies in Vernalisation of Cereals. II. The Vernalisation of Excised Mature Embryos and of Developing Ears'.

Fig. 1. Plants grown from excised embryos germinated on nutrient agar at 1° C. and at 18° C. Photographed ten weeks after planting.

Fig. 2. Method of vernalising ripening ears attached to the plants (see p. 240).

Fig. 3. Vernalisation in the ear. Plants in eight pots on left were grown from grain ripened on cut ears in water, and chilled during six weeks of the ripening period. Plants in eight pots on right were grown from grain similarly ripened but kept throughout at room temperature. Photographed ten and a half weeks after planting.





NOTES

With two Figures in the Text

A CONVENIENT METHOD FOR ATTACHING POTOMETERS AND AN EXAMPLE OF ITS USE IN MEASURING THE UPTAKE OF WATER BY LEAVES DURING RECOVERY FROM WILTING.—It is often necessary to effect rapidly connexion between plant stems or petioles and potometers in transpiration experiments. The following method has proved useful.

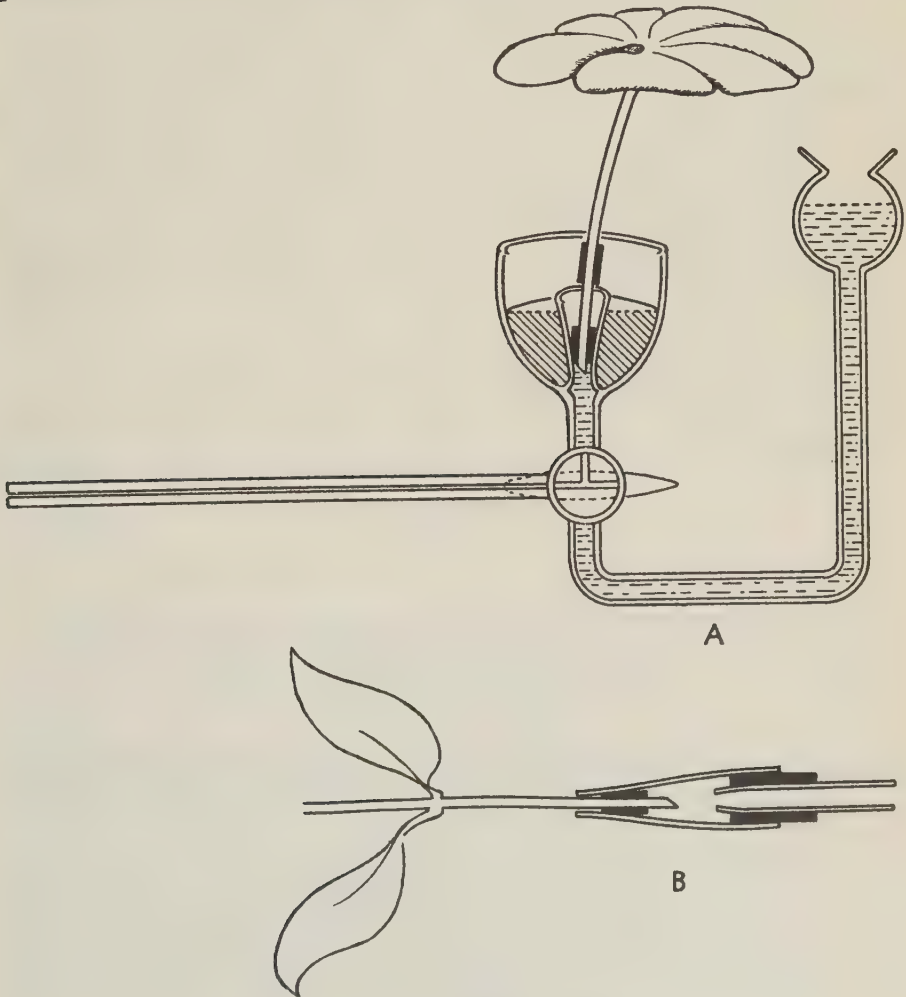


FIG. 1. For explanation see text.

The leaf or shoot is cut under water and a short length of valve rubber or wider thin rubber tubing is slipped on at the base some little distance above the cut surface, which is kept submerged. This can be performed by placing the rubber tubing on the end of a tube just wide enough to engage the plant tissue and then transferring the rubber to the desired position on the stem. The end of the shoot or

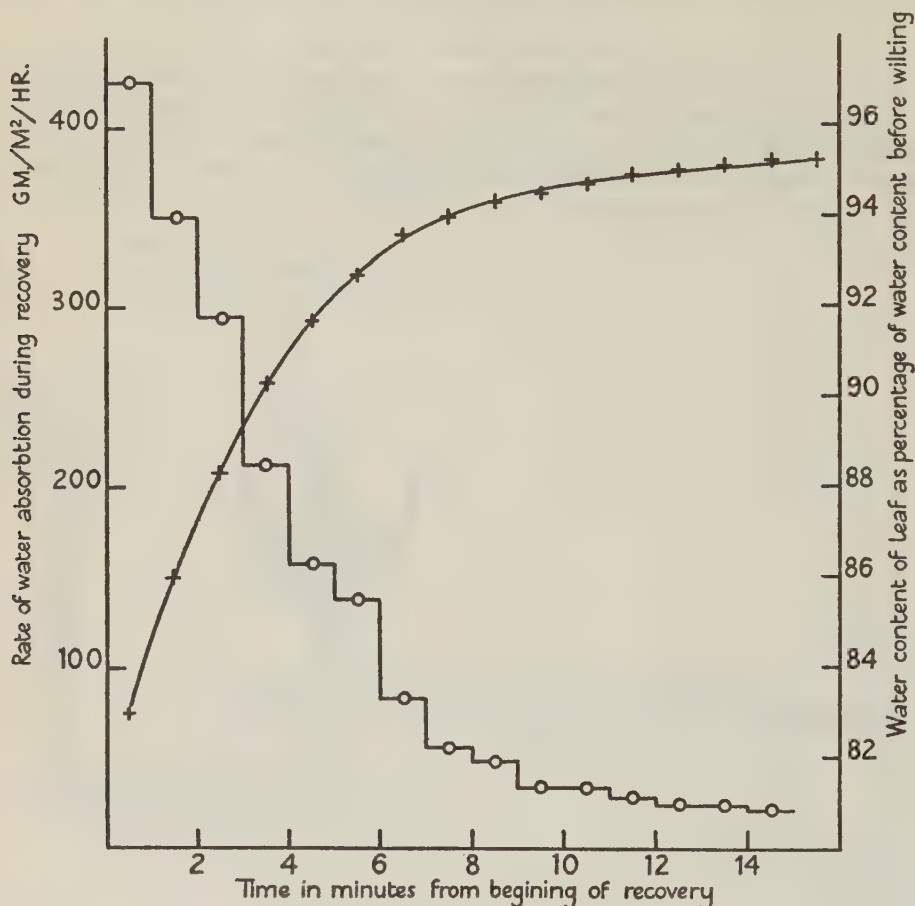


FIG. 2. For explanation see text.

petiole is then pushed into a conical glass tube of convenient taper so that the rubber is wedged into the cone, which is in turn connected to the potometer. The whole arrangement is shown diagrammatically in Fig. 1 A.

In transpiration experiments the suction pressure developed while the tap is closed tends to draw the material more firmly into the cone and ensures watertight connexion. In experiments in which positive pressures are to be applied to the cut end the whole arrangement is reversed so that the thrust of the water tends to compress the rubber against the glass. The necessary arrangement is shown in Fig. 1 B. The whole operation can be carried out in a very short time.

Many variations of this arrangement are possible, and one such is given below in

which the study of the uptake of water during recovery from wilting is described. In this experiment a single leaf is used, the petiole being fitted as described above with two rings of rubber tubing a convenient distance apart. The tapered cone is fused into a glass cup and below is attached through a three-way tap to the capillary tube serving as a potometer and to a supply of water for filling the latter. The arrangement is shown in Fig. 1A.

The transpiration rate of the leaf is first determined under the desired conditions and the annular space between the outer cup and the cone is filled with 5 per cent. agar, just above its solidification temperature. After shutting off the tap, the petiole is removed from the cone and the end immersed in the agar to just above the lower rubber ring. The agar sets almost immediately and this cuts off the water supply from the leaf. By allowing the leaf to remain thus for varying periods of time different degrees of water deficiency are obtained. When the recovery of the leaf is to be studied the surface of the solid agar is covered with a layer of water and the petiole is severed between the two rubber rings. The end of the petiole is now replaced in the cone, the tap turned on, and the water uptake again measured. The recovery is found to be extremely rapid. In leaves of *Pelargonium zonale* which have reached the stage of wilting water content returns to normal in about ten minutes. Data of such a recovery are presented graphically in Fig. 2. The stepped curve in the diagram shows the rate of absorption of water in successive minutes, after returning to water, by a leaf which has been deprived of water for forty minutes. The smooth curve shows for the same leaf the rise in water content during recovery from wilting. The plotted values represent the weight of water in the leaf at any time as percentage of the original weight of water just prior to wilting. The very rapid recovery is noteworthy.

A later communication will deal with the application of the method to the simultaneous study of change in water content, transpiration rate, water absorption, and stomatal aperture during and after wilting.

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SUDAN IV AS A MICROCHEMICAL TEST FOR FATS IN PLANT TISSUES.—In the course of certain investigations on the influence of storage on flours of different grades it was ascertained that the amount of fat or oil, i.e. the fraction soluble in petrol ether, and especially the amount of free unsaturated fatty acids present in this fraction, play an important part in the keeping quality of flours and also on the quality of the washed-out gluten. At one stage of these investigations it became necessary to find a suitable microchemical reagent for the presence of fats. Osmic acid was found to be unsuitable for a variety of reasons, while Sudan III was so erratic in its action that it had to be abandoned. British Drug Houses were consulted on the subject and they very kindly suggested the use of Sudan IV (toluene-azo-toluene-azo- β -naphthol) in place of Sudan III and referred me to a paper by Kay and Whitehead (*J. Path. and Bact.* 41, 305, 1935). These authors found that Sudan IV gave very reliable results as a microchemical test for fats in animal tissues when used under certain specific conditions. Although the Kay and

Whitehead technique works extremely well with animal tissues it was found necessary to modify it for plant tissues. If the conditions given below are rigidly adhered to it will be found that Sudan IV will give excellent and consistent results with plant tissues.

Preparation of reagent. A stock solution of Sudan IV is prepared by adding 2 gm. of the dye to a litre of absolute alcohol at room temperature, and then gently refluxing the mixture until all the powder has dissolved; the solution is then allowed to cool and settle. This stock solution keeps well.

For the staining of the sections, 7 parts of the stock solution are mixed with 9 parts of 45 per cent. alcohol. The sections to be examined (in this work the material was frozen in gum and then cut on a microtome) are placed in 50 per cent. alcohol for one and a half hours, and then transferred to the diluted Sudan IV solution and allowed to remain therein for *at least three hours at ordinary room temperature. The temperature should on no account be raised.* A longer immersion than three hours does no harm. The sections are then removed to 50 per cent. alcohol for three minutes, washed in distilled water for thirty minutes, counter-stained in Mayer's haemalum for at least three hours, washed in alkaline tap water and mounted in glycerine.

In the present investigation, a number of tests for fats were carried out on bran, which consists mainly of pericarp, testa, and aleurone layer, and also on the wheat grain itself. In the aleurone layer the fat is present in the form of large globules, and fat in the protoplasm can also be easily made out. The testa stains deeply with Sudan IV. After extraction with petrol ether the globules disappear, as is to be expected, but fat can still be made out in the protoplasm and the fat in the testa is not affected. It has been found by Rewald (*Chem. and Ind.* 55, 1002, 1936), and confirmed in this investigation, that it is possible to remove a second lipide fraction from wheat germ, as well as other parts of the grain, after preliminary extraction with petrol ether, by using a mixture composed of 80 parts of alcohol and 20 parts of benzene and extracting for thirty-six to forty-eight hours. By the use of this mixture it was found that protoplasmic fat was removed, but fat in the testa was unaffected. An examination of the wheat grain showed similar oil globules to be present in the aleurone layer as was the case in unextracted bran. No fat could be discovered in the endosperm, while in the embryo the plumule showed faint traces of fat; this appeared to increase towards the primary and secondary roots, but the root-caps were free of fat. The scutellum was heavily charged with fat as was also the epithelial layer. The fat in embryo, scutellum, and epithelial cells was found to be distributed evenly through the cell protoplasm and globules of fat were never observed. The globules appear to be entirely restricted to the aleurone layer of the grain.

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The Interaction of Light Intensity and Nitrogen Supply in the Growth and Metabolism of Grasses and Clover (*Trifolium repens*)

I. The Effects of Light Intensity and Nitrogen Supply on the Clover Content of a Sward

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With eight Figures in the Text

INTRODUCTION

NUMEROUS workers have recorded that in grassland communities the addition of nitrogenous compounds depresses the leguminous species. The investigations of Martin Jones (1933), and the author (1933), have demonstrated that this depression is largely dependent upon the frequency and severity of grazing. It has been shown that the amount of *Trifolium repens* in the sward can be maintained if the quantity of nitrogen added is not excessive and as long as the pasture is kept short by frequent defoliation during the spring and summer months. If on the other hand defoliation is infrequent and the herbage allowed to grow tall a fall in the clover content results, more particularly when nitrogen is added. The view is put forward by Könekamp and König (1929), and Stapledon and Milton (1932), that this reduction is largely a question of competition for light, the taller-growing grasses shading the clover. These workers suggest that the effect of nitrogen is to increase the degree of shading, since not only is the average height of the grasses increased but also the density.

It seemed probable at the time when these investigations were begun that, apart from competition for light, nitrogen supply might also play some part in controlling the clover-grass balance. In the first place on the basis of Prianishnikov's (1904, 1913, 1922) researches¹ the explanation has been advanced in a previous investigation (1934) that the reduction in clover content brought about in closely-cut turf by ammonium compounds was due to poisoning by ammonium ions. It was postulated that where very large quantities of ammonium salts were added to the soil, then the rapid uptake of

¹ For a fuller account of Prianishnikov's investigations reference should be made to the recent reviews of nitrogen metabolism by McKee (1937), and Nightingale (1937).

ammonium ions would exhaust the low concentration of available carbohydrates in the clover and lead eventually to the accumulation of free ammonium ions. If this hypothesis were substantially correct, it seemed probable that, where clover was shaded by the grasses, and its carbohydrate reserves kept low, then the addition of even small amounts of ammonium salts might lead to the toxic accumulation of free ammonium ions. Alternatively, if ammonium compounds were added in such amount as to cause a decrease of clover content without the indirect effect of shading, then the depression should be much increased under conditions of low light intensity. Since there was no evidence that the accumulation of nitrate ions was toxic even when little growth was taking place (Nightingale, 1927 and 1933; Ditttrich, 1931; Eckerson, 1932), it should follow on the same hypothesis, that the *difference* between ammonia-nitrogen and nitrate-nitrogen in their effect on the clover should be much greater under conditions of low light intensity.

It was with the object of investigating these possible interactions between nitrogen supply and light intensity that the present investigations were undertaken. This paper is confined to the results obtained in field experiments. The experiments were carried out on swards frequently defoliated, since it was known that such conditions were favourable to the development of *T. repens*. Variations in the nitrogen supply, were obtained by adding periodically either ammonium sulphate or calcium nitrate, and variations in light intensities secured by shading plots with suitable screens.

EXPERIMENTAL RESULTS

Experimental technique.

All the experiments were carried out at Jealott's Hill on swards frequently defoliated by a mowing machine. For the purpose of this investigation mowing had many advantages over grazing. In order to decrease the light intensity, plots were shaded with butter muslin, one or more layers thick, stretched on light wooden frames. In consequence the plot size was restricted to avoid very heavy and unwieldy frames. The necessarily small plot made grazing even by sheep impracticable. It would have introduced large errors, due to uneven grazing and the irregular distribution of droppings. Moreover, the time taken to graze the plots would have been much longer, and more variable than the time taken to cut them. In addition differences in light intensity between treatments could not have been maintained under grazing conditions within such exact limits. It was realized that cutting in contrast to grazing would bring about a more rapid depletion of the available nutrients in the soil. Nevertheless, even where cutting without replacement of the mineral substances removed in the herbage had been practised for many years, *T. repens* was one of the dominant plants in the sward. It was on this sward that the earlier experiments of 1933 and 1934 were laid out.

In 1933 and 1934 the replication of each treatment was fourfold. Since on the experimental area the distribution of clover was very variable each experi-

ment of those years was not laid out in blocks. Instead the requisite number of plots containing an approximately equal amount of clover were first chosen and individual plots allotted at random to the several treatments. In 1935 and 1936, when different swards with a more even distribution of clover were used, the experiments consisted of random blocks in three—or fourfold replication.

The method of estimating the changes in clover content has been described previously (1932). Briefly the method consists in determining by eye the percentage area covered within a number of small quadrats (6 in. $\times$ 6 in.). For greater accuracy in estimation the quadrat is divided by thin wires into a number of smaller areas each 2 to 4 sq. in. In 1933–5 ten quadrats were chosen per plot. In 1936 forty quadrats of 2 sq. in. were used instead. Estimates of the clover content were made every seven days during the experimental period.

As has already been stated, variations in the light intensity were controlled by shading the plots with butter muslin stretched on wooden frames. Preliminary trials were made with frames of two sizes, those large enough to cover plots 6 ft. $\times$ 6 ft. or 4 ft. $\times$ 2 ft. In the larger frames a single thin cross-batten was added with the object of strengthening the frame and preventing the butter muslin sagging in the middle; on the smaller frame there was no cross-batten. The larger frame was eventually discarded as unwieldy, and because, during bad weather the muslin tended to tear. Also in heavy rain the wet material always sagged to some extent so that the middle of the plot received more than its due proportion of water. In the smaller frames there was little tendency for the material to sag, the muslin did not tear, and the frames were easy to handle. The frames were kept in position by means of four galvanized iron right-angle brackets. One arm of the bracket was pressed into the soil, the other arm was screwed into the frame at the corner. Between the bottom of the frame, and the soil surface there was a clearance of 1–2 in. in order to increase the circulation of air.

During 1933 estimates of the degree of shading were made by testing the material in the laboratory by a 'Holophane' lumeter. In 1934–6 the amount of light transmitted was estimated *in situ* by means of a 'Weston' phototronic cell coupled to a 'Ferranti' milliammeter. The resistance in the circuit was adjusted so that between 0 and 2,000-ft. candles the current generated by the cell was directly proportional to the light intensity. In these latter experiments a large number of readings were taken from time to time during the run of each experiment.

When either ammonium sulphate or calcium nitrate was applied to the appropriate plots, it was added in dilute solution. As a further precaution in order that the herbage should not be damaged by solutions of high osmotic concentration, the plots were subsequently watered. In 1933 5 where ammonium sulphate had been added, calcium carbonate was applied a few days later in order to counteract any tendency for the soil to become acid.

Subsequent to 1933 in addition to each plot receiving weekly 2 gallons of water (equivalent to 0.5 in. rain) at the time of each fertilizer application the plots were further watered during dry weather.

Effect of nitrogen supply and shading on clover content.

1933 data. In 1933-4 the experiments were carried out on a sward, which had been cut for at least ten years with a mowing machine, and as far as is known had received no manurial treatment during that time. The sward consisted very largely of *Agrostis tenuis* and *T. repens*, the commonest occasional species being *Festuca ovina* and *Prunella vulgaris*. In the 1933 'balanced'

TABLE I

Changes in Clover Content in Relation to Light Intensity and Nitrogen Supply.
(Statistical analysis based on regressions of rate of diminution against time.)
1933 Experiment 1. July 20 to Sept. 6

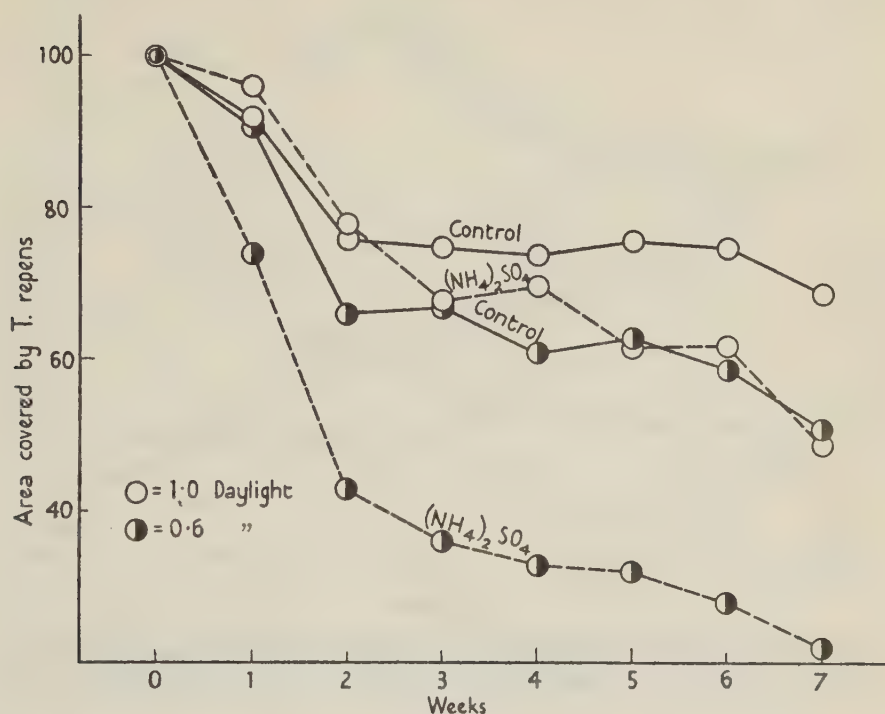
Light Treatments				Manurial Treatments		Mean.
				Control.	(NH ₄) ₂ SO ₄ .	
	1.0 daylight	.	.	0.0144	0.0295	0.0220
	0.6 daylight	.	.	0.0297	0.0511	0.0404
	Mean	.	.	0.0220	0.0403	—
Significant difference between treatments					= 0.0294	
Significant difference between means of 2 treatments					= 0.0208	
(P = 0.05)						

experiment there were four treatments, two light treatments and two manurial treatments. Half the plots received ammonium sulphate every seven days, at the rate of 37.5 lb. nitrogen per acre; the other half received no nitrogen. Half the plots (one quarter with and one quarter without nitrogen), were shaded with a single layer of butter muslin, while the other half were unshaded. Measurement of the proportion of light transmitted by the muslin gave an average figure of 60 per cent.

The results of this experiment are shown graphically in Fig. 1, while the statistical analysis of the data is given in Table I. In Fig. 1, and in all subsequent figures it has been found useful to take the initial clover content of each treatment as 100, and express any subsequent change relative to this figure. In the statistical analysis no attempt was made to analyse the percentage area figures direct, since it had been shown in a previous investigation (Blackman, 1935), that the distribution curves of such data are often markedly skew. It was decided, therefore, to fit a linear regression (change in percentage area covered against time) to the data for each plot, and analyse statistically the regression figures. Inspection of the original data indicated, that in this, and the following experiments linear regressions with smaller errors could be obtained by transposing the data. For x (the percentage area covered) y was substituted on the basis that y equals $\log \frac{x}{100-x}$. The data transposed in this

way were worked out by the analysis of covariance with time. Subsequently, to obtain the regression coefficients, the data were divided throughout by the sum of the squares for time.

Inspection of Fig. 1, and the data given in Table I shows that neither the



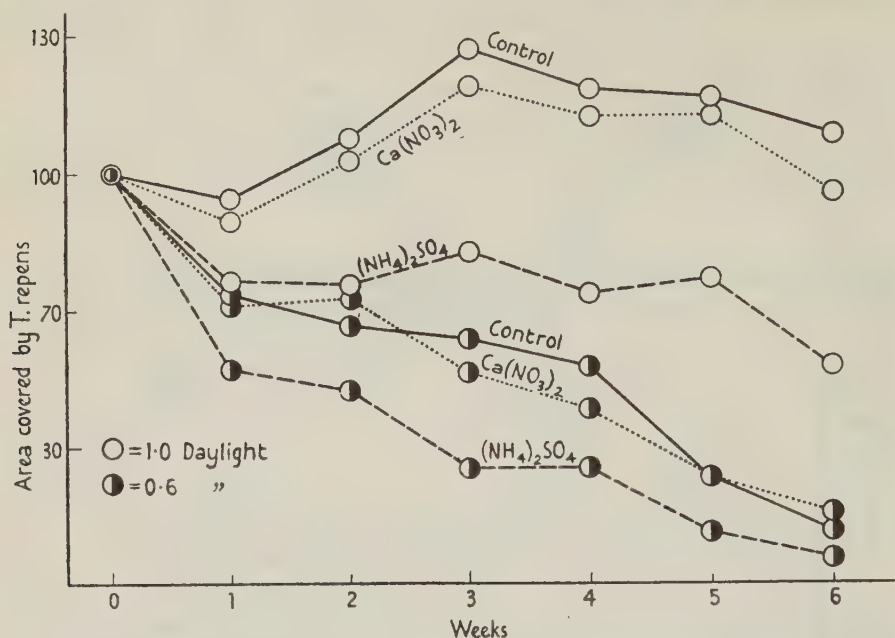
TEXT-FIG 1. The effects of light intensity and nitrogen supply on the clover content of a sward. For each treatment the changes in area covered are expressed relative to the initial content (27.6–29.7 per cent.) which has been taken as 100. (1933. Experiment 1.)

addition of ammonium sulphate, nor the shading of the herbage significantly reduced the clover content. On the other hand the combination of these two factors brought about a significant reduction.

1934 data. In 1934 the experiments were carried out on the same sward as in 1933. In both these experiments there were two additional treatments as calcium nitrate was included in addition to ammonium sulphate. The rate and frequency of the nitrogen application was the same as in 1933. For reducing the light intensity a single layer of butter muslin was again used and the percentage of light falling on the sward was estimated as 60 per cent. of daylight.

The results of the first experiment carried out during 1934 are seen in Fig. 2 and Table II. Under conditions of high light intensity, ammonium sulphate in contrast to calcium nitrate significantly decreased the clover, but under the low light intensity no nitrogen effect appeared. Reduction of light intensity brought about a marked fall in the clover irrespective of the

nitrogenous manuring. Taking both light treatments into account ammonium sulphate was significantly more effective than calcium nitrate in suppressing the clover.



TEXT-FIG. 2. The effects of light intensity and nitrogen supply on the clover content of a sward. For each treatment the changes in area covered are expressed relative to the initial content (30.2–31.7 per cent.), which has been taken as 100. (1934. Experiment 1.)

TABLE II

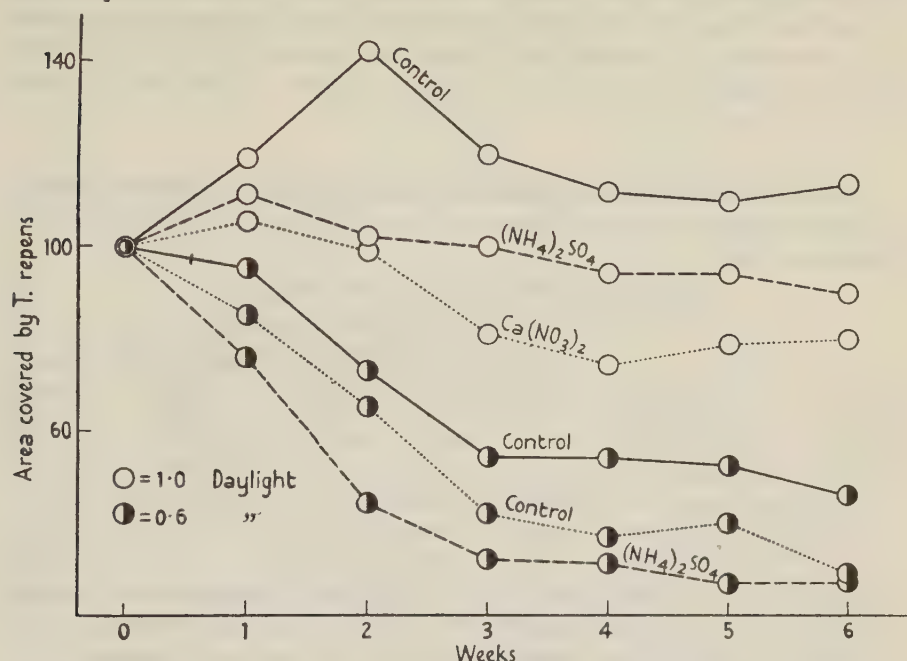
Changes in Clover Content in Relation to Light Intensity and Nitrogen Supply.
(Statistical analysis based on regressions of rate of diminution against time. For details see p. 260.)

1934 Experiment 1. June 21 to Aug. 3

		Manurial treatments			Mean.
		Control.	$(\text{NH}_4)_2\text{SO}_4$	$\text{Ca}(\text{NO}_3)_2$.	
Light Treatments	1.0 daylight	—0.004	0.046	0.001	0.014
	0.6 daylight	0.133	0.153	0.129	0.138
	Mean	0.065	0.099	0.065	—
Significant difference between treatments					= 0.048
Significant difference between means of 2 treatments					= 0.032
Significant difference between means of 3 treatments					= 0.026
(P = 0.05)					

The results of the second experiment of 1934 are given in Fig. 3, and Table III. During the course of this experiment there was a period of abundant rain and two of the plots which showed fungal attack had to be discarded.

As a result it was necessary in the statistical analysis to estimate the regressions for the 'missing' plots. In consequence the error of the experiment was considerably increased.



TEXT-FIG. 3. The effects of light intensity and nitrogen supply on the clover content of a sward. For each treatment the changes in area covered are expressed relative to the initial content (21.8–24.0 per cent.), which has been taken as 100. (1934. Experiment 2.)

TABLE III

Changes in Clover Content in Relation to Light Intensity and Nitrogen Supply.
(Statistical analysis based on regressions of rate of diminution against time. For details see p. 260.)

1934 Experiment 2. Aug. 3 to Sept. 13

		Manurial treatments			Mean.
		Control.	$(\text{NH}_4)_2\text{SO}_4$.	$\text{Ca}(\text{NO}_3)_2$.	
Light Treatments	1.0 daylight . . .	0.0021	0.0186	0.0422	0.0210
	0.6 daylight . . .	0.0829	0.1127	0.1100	0.1019
	Mean . . .	0.0425	0.0657	0.0761	—
Significant difference between treatments					= 0.0577
Significant difference between means of 2 treatments					= 0.0408
Significant difference between means of 3 treatments					= 0.0333
(P = 0.05)					

As in the first experiment of 1934, reducing the light intensity brought about a marked diminution in the clover content with or without the addition of nitrogen. On the other hand none of the nitrogen effects were significant.

1935 data. The experimental sites were different from those of previous years. Three experiments (experiments 1–3) were laid down on a specially levelled area, which had been sown five years previously with a mixture of *A. tenuis* and *F. rubra*. By 1935 *F. rubra* had been completely suppressed, and under the system of constant cutting, the area had been invaded by *T. repens* and *Poa trivialis*. When the experiments were begun *T. repens* and *A. tenuis*

TABLE IV

Changes in Clover Content in Relation to Light Intensity and Nitrogen Supply. (Statistical analysis based on regressions of rate of diminution against time. For details see p. 260.)

1935 Experiment 1. July 9 to Aug. 6

		Manurial treatments			Mean.
		Control.	(NH ₄) ₂ SO ₄ .	Ca(NO ₃) ₂ .	
Light Treatments	1.0 daylight . . .	—0.0923	0.0477	0.0207	—0.0080
	0.6 daylight . . .	0.0277	0.1347	0.0900	0.0841
	0.4 daylight . . .	0.0643	0.1377	0.0917	0.0979
	Mean . . .	—0.0001	0.1067	0.0674	—
Significant difference (i) between treatments				= 0.0552	
Significant difference (ii) between means of 3 treatments				= 0.0319	
(P = 0.05)					

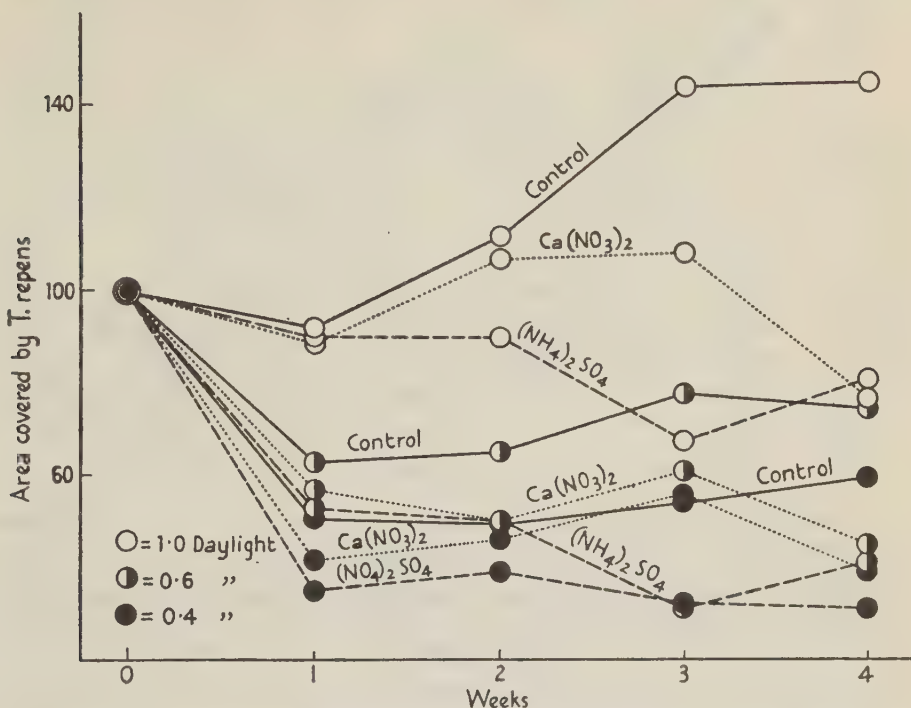
were the dominant plants in the sward. The fourth experiment was carried out on another levelled area, which for the purpose of this investigation was sown in the autumn of 1934, with a mixture of *A. tenuis* and *T. repens*.

Each experiment consisted of nine treatments in all; ammonium sulphate, or calcium nitrate was applied at the rate of 50 lb. nitrogen per acre, every seven days as against 37.5 lb. in the previous experiments. There were in addition three levels of light intensity; some plots were shaded with a single layer of butter muslin (60 per cent. of daylight), and others with a double layer (40 per cent.).

The results set out in Table IV and Fig. 4 show that at all three levels of light intensity the addition of ammonium sulphate significantly increased the rate of clover diminution. Calcium nitrate produced similar results except at the lowest light intensity. The average effect of ammonium sulphate on the clover content was significantly greater than that of calcium nitrate. Decreasing the light intensity from daylight to 0.6 daylight markedly reduced the clover. In contrast a further decrease in intensity to 0.4 daylight had no effect.

Experiments 2 and 3 were carried out at the same time and on the same sward. The results, however, have been analysed separately since the initial clover content of the two experimental areas were not equal, i.e. 34.2 per cent. of the area was covered by *T. repens* in experiment 2, and 26.2 per cent. in experiment 3. Although the magnitude of the changes was greater in experiment 2 (see Fig. 5 and Table V), than in experiment 3 (see Fig. 6 and Table

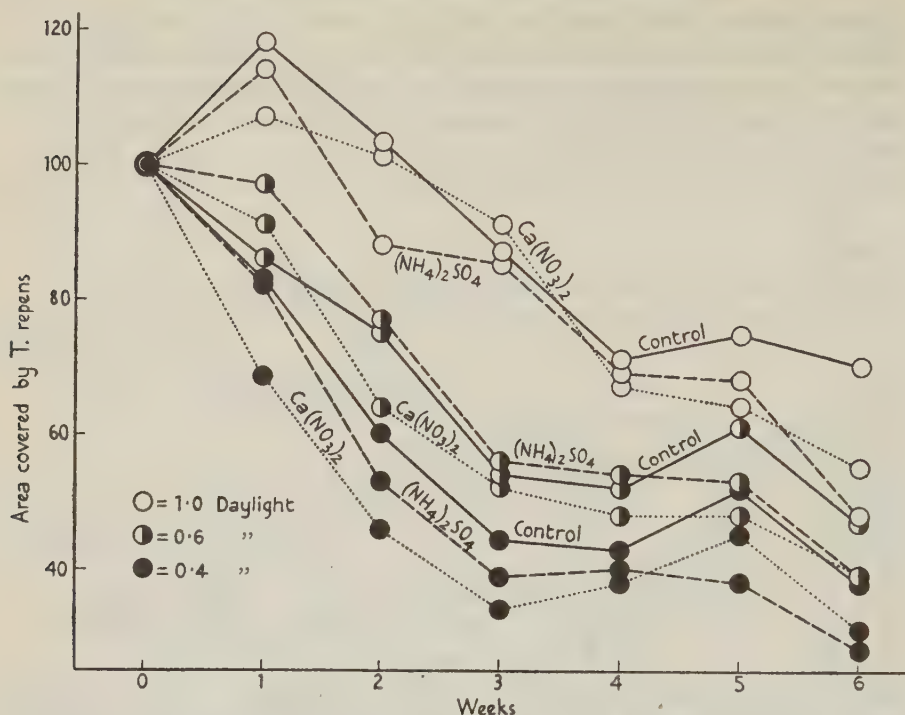
VI), the general trend was similar. In both experiments at the highest light intensity neither ammonium sulphate nor calcium nitrate significantly suppressed the clover, though in experiment 3 the effect of ammonium sulphate was only just not significant. Reducing the daylight to 0.6 diminished the



TEXT-FIG. 4. The effects of light intensity and nitrogen supply on the clover content of a sward. For each treatment the changes in area covered are expressed relative to the initial content (34.9–37.8 per cent.), which has been taken as 100. (1935. Experiment 1.)

clover content irrespective of the changes due to manurial treatments, but a further decrease in light intensity to 0.4 had no further effect. Considering the manurial treatments apart from light it is found that ammonium sulphate in both experiments significantly decreased the clover while calcium nitrate did not.

In regard to differences between individual treatments the experiments were somewhat dissimilar. In experiment 2 the reductions in light intensity did not significantly affect the clover content where no nitrogen was added, but in experiment 3 the differences were significant. In both experiments 2 and 3 ammonium sulphate at the lowest light intensity decreased the clover more than at the highest light intensity, but at the intermediate level the results were not significant. Calcium nitrate on the other hand showed in experiment 2 no relationship with light intensity. In experiment 3 decreasing the light to 0.6 accelerated the rate of clover diminution, but a further decrease to 0.4 retarded the rate of diminution.



TEXT-FIG 5. The effects of light intensity and nitrogen supply on the clover content of a sward. For each treatment the changes in area covered are expressed relative to the initial content (32.6–38.0 per cent.), which has been taken as 100. (1935. Experiment 2.)

TABLE V

Changes in Clover Content in Relation to Light Intensity and Nitrogen Supply.
(Statistical analysis based on regressions of rate of diminution against time. For details see p. 260.)

1935 Experiment 2. Aug. 13 to Sept. 23

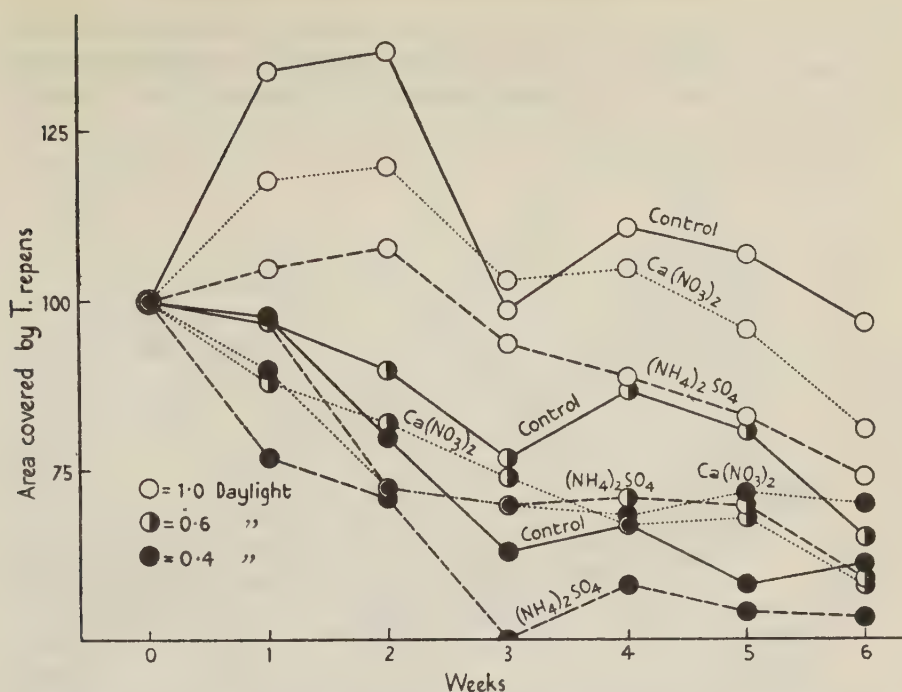
		Nitrogen treatments			Mean.
		Control.	(NH ₄) ₂ SO ₄ .	Ca(NO ₃) ₂ .	
Light Treatments	1.0 daylight . . .	0.0546	0.0719	0.0698	0.0654
	0.6 daylight . . .	0.0707	0.0879	0.0888	0.0825
	0.4 daylight . . .	0.0820	0.1111	0.0886	0.0939
	Mean . . .	0.0691	0.0903	0.0824	—

Significant difference (i) between treatments = 0.0282

Significant difference (ii) between means of 3 treatments = 0.0163

(P = 0.05)

The last experiment of 1935 was carried out in the autumn on the sward, which had been sown some twelve months earlier with a mixture of *A. tenuis* and *T. repens*. The results of this experiment are seen in Table VII and Fig. 7. In full daylight the addition of both ammonium sulphate, and calcium nitrate



TEXT-FIG. 6. The effects of light intensity and nitrogen supply on the clover content of a sward. For each treatment the changes in area covered are expressed relative to the initial content (24.1–27.3 per cent.), which has been taken as 100. (1935. Experiment 3.)

TABLE VI

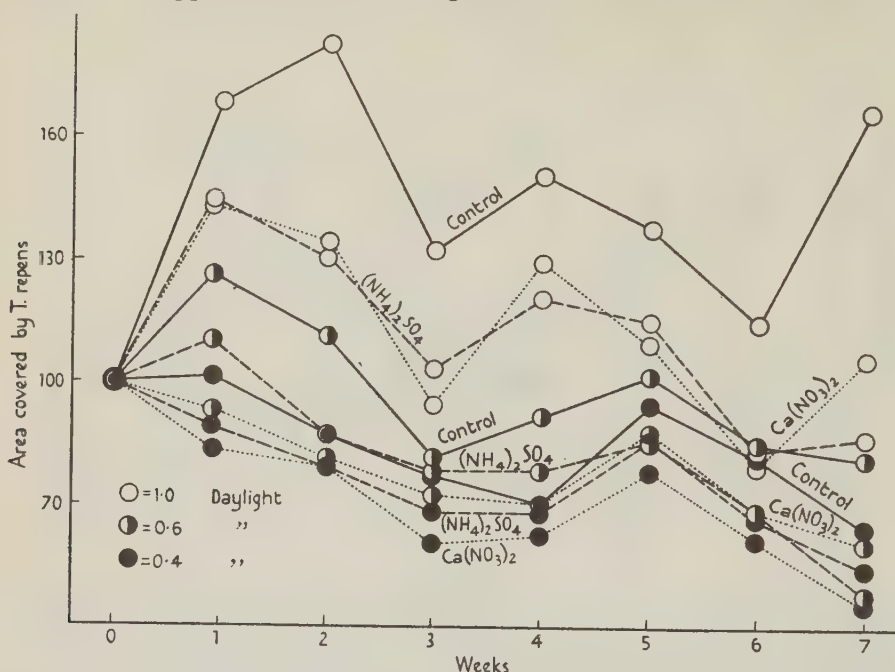
Changes in Clover Content in Relation to Light Intensity and Nitrogen Supply. (Statistical analysis based on regressions of rate of diminution against time. For details see p. 260.)

1935 Experiment 3. Aug. 13 to Sept. 23

		Nitrogen treatments			Mean.
		Control.	(NH ₄) ₂ SO ₄ .	Ca(NO ₃) ₂ .	
Light Treatments	1.0 daylight . . .	0.0162	0.0324	0.0254	0.0246
	0.6 daylight . . .	0.0339	0.0471	0.0426	0.0412
	0.4 daylight . . .	0.0535	0.0529	0.0283	0.0449
	Mean . . .	0.0345	0.0441	0.0321	—
Significant difference (i) between treatments		= 0.0165			
Significant difference (ii) between means of 3 treatments		= 0.0095			
(P = 0.05)					

brought about a significant reduction in the clover content. At the lower light intensities the nitrogen treatments (except ammonium sulphate at 0.6 daylight) did not significantly increase the rate of reduction. Nevertheless the average effect of both ammonium sulphate and calcium nitrate at the three light levels was to suppress significantly the clover. Lowering the light

intensity from 1.0 to 0.6 brought about a significant reduction in the clover content, more particularly where no nitrogen was given, or where ammonium sulphate was applied. At the lowest light level there was no further effect.



TEXT-FIG. 7. The effects of light intensity and nitrogen supply on the clover content of a sward. For each treatment the changes in area covered are expressed relative to the initial content (17.1–19.7 per cent.), which has been taken as 100. (1935. Experiment 4.)

TABLE VII

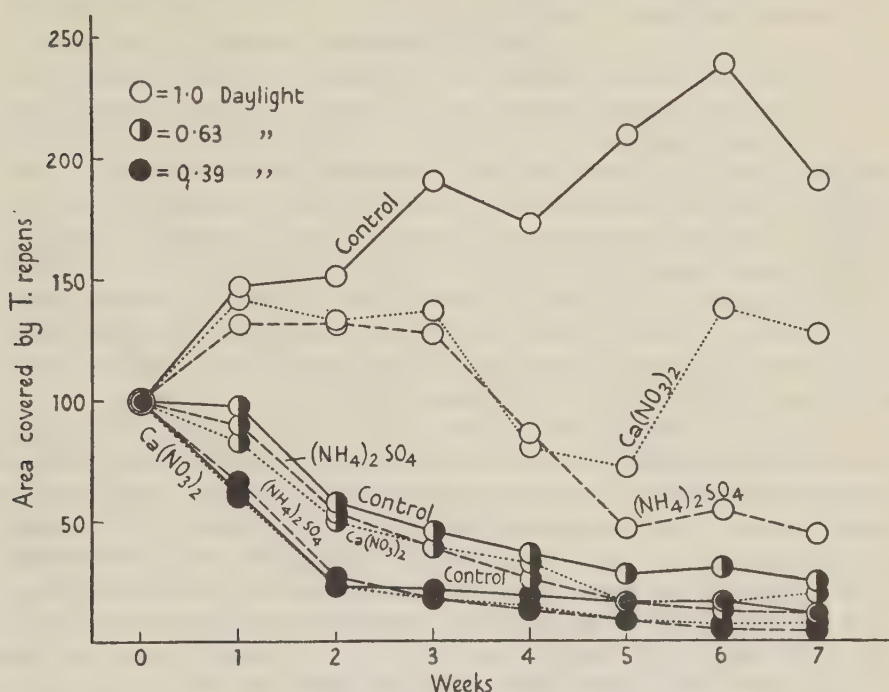
Changes in Clover Content in Relation to Light Intensity and Nitrogen Supply.
(Statistical analysis based on regressions of rate of diminution against time. For details see p. 260.)

1935 Experiment 4. Aug. 13 to Oct. 3

		Nitrogen treatments			Mean.
		Control.	(NH ₄) ₂ SO ₄ .	Ca(NO ₃) ₂ .	
Light Treatments	1.0 daylight	—0.0016	0.0132	0.0095	0.0070
	0.6 daylight	0.0117	0.0239	0.0149	0.0168
	0.4 daylight	0.0130	0.0172	0.0208	0.0170
	Mean	0.0077	0.0181	0.0151	—
Significant difference (i) between treatments		= 0.0109			
Significant difference (ii) between means of 3 treatments		= 0.0063			
(P = 0.05)					

1936 data. In the spring of 1936 an experiment was carried out on a site adjacent to that of the experiment laid down in the previous autumn on the sward originally sown with a mixture of *A. tenuis* and *T. repens*. In design the

experiment was similar to those of 1935, there were three levels of light intensity, while the ammonium sulphate and calcium nitrate were again applied at the rate of 50 lb. N. per acre. The results of this experiment are seen in Table VIII and Fig. 8. With all nitrogen treatments a reduction in the daylight intensity from 1.0 to 0.63 significantly decreased the clover, while



TEXT-FIG. 8. The effects of light intensity and nitrogen supply on the clover content of a sward. For each treatment the changes in area covered are expressed relative to the initial content (18.7–25.1 per cent.), which has been taken as 100. (1936. Experiment 1.)

TABLE VIII

Changes in Clover Content in Relation to Light Intensity and Nitrogen Supply.
(Statistical analysis based on regressions of rate of diminution against time. For details see p. 260.)

1936 Experiment 1. May 11 to June 30

		Nitrogen treatments			Mean.
		Control.	(NH ₄) ₂ SO ₄ .	Ca(NO ₃) ₂ .	
Light Treatments	1.0 daylight . . .	—0.0297	0.0415	0.0028	0.0049
	0.63 daylight . . .	0.0525	0.0862	0.0697	0.0695
	0.39 daylight . . .	0.0694	0.1141	0.0922	0.0919
	Mean . . .	0.0308	0.0806	0.0549	—

Significant difference (i) between treatments = 0.0173

Significant difference (ii) between means of 3 treatments = 0.0100

(P = 0.05)

a further reduction to 0.39 caused a further depression. At all three levels of light the addition of ammonium sulphate suppressed the clover; at the lowest and highest levels this reduction was significantly greater than that brought about by calcium nitrate. Calcium nitrate also diminished the clover both in full light and in 0.39 light.

Influence of additional nitrogen on soil reaction. In all the experiments the surface layers of the soil were initially approximately neutral. It was realized, that while the addition of calcium nitrate would not affect appreciably the hydrogen ion concentration, ammonium sulphate would tend to make the soil acid. In order to eliminate this difference between the two forms of nitrogen, subsequent to each application of ammonium sulphate, calcium carbonate was applied a few days later at the rate of 85 gm. for every 100 gm. of ammonium sulphate. This ratio was used since it had been found previously at Jealott's Hill (Blackman, 1934), that under these conditions the soil reaction was maintained at the initial level. At the conclusion of the experiment, cores to a depth of three inches were taken from each plot and divided into three equal transverse sections. The samples were air dried and the pH determinations carried out by the quinhydrone method, using gold electrodes.

The results obtained are in agreement with the previous findings, namely, that little if any acidity develops when calcium carbonate is added with ammonium sulphate. In none of the 1933–5 experiments were the differences in pH between treatments greater than 0.5. Even in the 1936 experiment, when calcium carbonate was not added, the final differences in pH between the soils treated with either ammonium sulphate or calcium nitrate amounted only to 0.93 in the first inch, 0.59 in the second, and 0.36 in the third.

Since in all the experiments the differences in the soil reaction were so small, it seems highly improbable that they played any important part in bringing about the changes in clover content observed.

Meteorological observations. Mention has already been made of the differences in rainfall occurring during the various experimental periods. In Table IX the average daily rainfall together with other relevant data have been set out. The soil temperature at a depth of 4 in. (9 a.m. determination), has been taken as the most suitable single measure of temperature changes. The hours of sunshine were measured by means of a Campbell-Stokes recorder.

The data show that in two experiments (1933, expt. 1; 1935, expt. 1), the rainfall was much below the average. In the 1933 experiment, when the plots were not watered, water undoubtedly was limiting growth in the unshaded plots. The failure of shading to reduce the clover content (see Table I) may, therefore, have been due to water, not light, controlling growth, even though the loss of water by transpiration and evaporation was probably restricted to some extent by shading. In the 1935 experiment the water factor was unlikely to have been operative, since in this experiment the plots received weekly additional water equivalent to 0.5 to 1.0 in. of rain.

There does not seem to be any direct relationship between the effects of

reduced light intensity on the clover content and the absolute amount of light received during the day. The magnitude of the depression caused by a reduction in the light intensity to 0.6–0.63 daylight is in no way correlated either, with hours of sunshine, or length of day. In fact in experiments 2–4 of 1935

TABLE IX
Meteorological Data

Expt. no.	Soil temperature 4 in. depth, ° F.		Rainfall, in. per day.		Sunshine, hrs. per day.		Length of day. hrs. from sunrise to sunset.
	Mean.	Standard deviation.	Mean.	Standard deviation.	Mean.	Standard deviation.	
1933 expt. 1	66.3	2.74	0.008	0.024	8.1	3.48	14.78
1934 „ 1	63.9	3.04	0.066	0.139	7.8	4.97	16.24
„ „ 2	59.1	2.59	0.085	0.178	6.5	3.58	14.38
1935 „ 1	66.7	2.24	0.011	0.028	8.7	4.03	15.94
„ „ 2	60.9	3.62	0.113	0.186	5.4	4.08	13.61
„ „ 3	60.9	3.62	0.113	0.186	5.4	4.08	13.61
„ „ 4	59.7	4.25	0.142	0.209	5.0	3.96	13.40
1936 „ 1	59.5	4.99	0.058	0.112	6.6	4.64	16.08

the suppression of the clover (see Tables V to VII) is less pronounced than in experiment 1 (Table IV), or in the 1934 and 1936 experiments (Tables II, III, and VIII). Moreover, in the single case, where a further reduction of the light intensity to 0.39 depressed still more the clover content (1936 experiment 1), the hours of sunshine were neither the lowest nor the length of day the shortest. However the effects of insolation cannot be separated from those of temperature. Since temperature is necessarily correlated both with hours of sunshine and length of day, shading, as would be expected, brought about differences in temperature between the unshaded and shaded plots. A reduction in light intensity although unlikely to affect the air temperature under the experimental conditions, undoubtedly diminished the direct heating effect of both the soil and the herbage. Conversely during the night, the screens decreased the rate at which heat was lost. Soil temperature records taken at 9 a.m. and 5 p.m. at a depth of 4 in. demonstrated this change in the diurnal cycle of temperature. For example in experiment 1, 1935, which showed the most marked differences on account of the high average insolation, reductions of light intensity to 0.6 and 0.4 of daylight decreased the soil temperature by 0.63 and 0.8° F. at 5 p.m., but increased the temperature by 0.44 and 0.6° F. at 9 a.m. Although at a depth of 4 in. these differences were not very marked, undoubtedly nearer the surface the discrepancies were greater. Nevertheless, it is not apparent that such differences in temperature played much part in determining the effect of light intensity and nitrogen supply on the clover content. For example in 1935, experiments 2 and 3 were carried out during the same period on adjacent areas yet the changes in clover content were in many respects dissimilar (see p. 265).

DISCUSSION

From the results of this investigation it is possible to draw some general conclusions as to the effect on the clover content of variations in light intensity, and in nitrogen supply. A lowering of the daylight level to 0.6 and 0.63 has, in the absence of extra nitrogen, significantly depressed the clover content in six out of the eight experiments. Where ammonium sulphate was added the decrease in clover content due to the lower light level, was only significant in half of these eight experiments. In the seven experiments where calcium nitrate was also applied, the same decrease in light intensity suppressed the clover significantly in five experiments. Considering the light effect apart from the nitrogen factor it is found that in only one of the eight experiments was the diminution of clover not significant. Although diminishing the light intensity to 0.6–0.63 had such a marked effect, a further reduction to 0.39–0.4 did not on the whole show a progressive influence. In the five experiments of 1935–6, the average effect of lowering the light intensity from the intermediate to the low level was alone significant in the 1936 experiment.

As to the effect of additional nitrogen on the clover content, the results obtained in normal daylight are in agreement with previous findings (Blackman, 1934). The addition of nitrogen tended to suppress the clover; ammonium sulphate caused a significant depression in four out of the seven experiments of 1934–6, calcium nitrate in three. In six of these experiments the rate of diminution brought about by ammonium sulphate was greater than that effected by calcium nitrate; but in only two experiments can the difference be considered significant. At the light level of 0.6–0.63 increasing the nitrogen supply was less effective in suppressing the clover than with full daylight. In only three experiments was the depression caused by ammonium sulphate significant, and there were only two significant decreases where calcium nitrate was added. At this light level in none of the seven experiments was the difference between ammonium sulphate and calcium nitrate significant.

At the lowest light intensity (0.39–0.4) a significant decrease in clover was obtained with ammonium sulphate in three out of the five experiments, but in only one experiment was the effect of calcium nitrate significant. In contrast with the results obtained at the intermediate light level, the difference between ammonium sulphate and calcium nitrate was significant in two out of the five experiments. Finally, considering the average effect of additional nitrogen over the range of the three light intensities, ammonium sulphate brought about a significant decrease in six out of the seven experiments of 1934–6, and calcium nitrate in four only. In four experiments, the decrease in clover caused by ammonium sulphate was significantly greater.

In seeking to interpret the results of this investigation it seems proper to draw a distinction between the direct and indirect effects on the clover. Variations of light intensity, and of nitrogen supply may act directly by controlling its growth; they may also act indirectly through their effects on the

growth of the grasses in competition with the clover. Moreover, differences in the levels of light intensity and nitrogen supply may alter not only the intensity of this competition, but also its nature.

In the hypothesis stated earlier in this paper it was assumed that a decrease in light intensity diminished both the growth and the available carbohydrate supply of the clover. Under these conditions therefore, ammonium sulphate and calcium nitrate should have divergent effects. At the lower light levels ammonium sulphate should bring about greater decreases in the clover content than at the high level, since with a deficiency of available carbohydrates the absorbed ammonium ions should more readily reach a toxic concentration. On the same hypothesis the effect of calcium nitrate should not be related to the light intensity since the accumulation of nitrate ions would not be deleterious. The findings stated in the body of this paper are not, however, in agreement with this hypothesis.

The results show that a decrease of light intensity diminishes the clover content. It is considered that this must be a direct effect on the clover unless one assumes that the reduction of the light intensity increases the growth of the grasses. This supposition is highly improbable since in full daylight the marked response of the sward to the added nitrogen (see also Blackman, 1934) indicated that the growth of the grasses was limited by nitrogen supply. A reduction in clover at low light levels must therefore be associated with low carbohydrate reserves.¹ It would be expected therefore, that the effect of ammonium sulphate would be greater, the lower the light intensity. Yet this is not the case. Taking the averages for the years 1934-6 the differences between the regressions of the control and ammonium sulphate series are 0.0447 and 0.0323 for full daylight and for 0.6-0.63 daylight respectively. Similarly, the figures for the five experiments of 1935-6 are 0.0519, 0.0377, 0.0302 at the light levels of 1.0, 0.6-0.63 and 0.39-0.4. Evidently the lower the light intensity the less effective is the addition of ammonium sulphate in decreasing the clover. Comparable data for calcium nitrate contrary to the hypothesis show the same trend. Differences between the regressions for the control and calcium nitrate series for the three light levels (1.0, 0.6-0.63, 0.39-0.4 of daylight) are on an average 0.0362, 0.0229, 0.0079. Since both ammonium sulphate and calcium nitrate behave similarly, a reduction in the light intensity does not as was postulated accentuate the differences between ammonia- and nitrate-nitrogen. The experiments, therefore, in no way bear out the view that the greater reduction of clover brought about by ammonium sulphate is primarily due to a toxic accumulation of ammonium ions.

Apart, however, from any toxic action of ammonium ions nitrogenous manuring, with either ammonium sulphate or calcium nitrate, may directly bring about a physiological disturbance of the clover through its effect on nitrogen fixation. During the last twenty years a large number of workers

¹ That reducing the light intensity to 0.4 of daylight decreases both the growth and carbohydrate content of *T. repens* and some grasses has been demonstrated in other experiments.

have investigated the relationship between nodule formation and external inorganic nitrogen supply. It is not proposed to discuss these investigations in detail since the whole subject of legume symbiosis has recently been reviewed by Allison (1935) and Wilson (1935, 1937). It has been demonstrated by many workers, that a high external concentration of inorganic nitrogen inhibits the formation of nodules. In addition, excess of inorganic nitrogen reduces fixation of nitrogen in the nodules already formed (Hopkins *et al.*, 1932; Thornton and Rudolf, 1936). Hopkins *et al.* have recorded that even at concentrations of nitrate-nitrogen as low as 20–30 p.p.m. nodule formation is retarded in *T. pratense*, while 160 parts per million is sufficient to suppress more or less completely nitrogen fixation. In the present investigation nitrate-nitrogen has been added in amounts equivalent to a concentration of 50–67 p.p.m. in the top 3 in. of oven-dry soil, i.e. the region of the nodule bearing roots. In terms of soil water the concentration would be much higher, depending upon the soil-moisture content. On a conservative estimate this initially would be at least three times the concentration based on the soil weight, i.e. 150–171 p.p.m. It is clear, therefore, that the concentration of nitrate-nitrogen is at least high enough to interfere with nodule formation if not to suppress nitrogen fixation. Under these conditions clover is in active competition with the grasses for either the whole or part of its nitrogen supply.

Lemmermann (1907) has put forward the view, supported by Loehwing (1937), that leguminous plants are unable to absorb inorganic nitrogen as rapidly as the grasses, and therefore, tend to be suppressed, when nitrogen is not obtained by fixation. Evidence on the rate of absorption of inorganic nitrogen by legumes is scanty. Both Allison (1935), and Thornton (1936), consider that inorganic nitrogen can be absorbed fast enough to upset the carbon-nitrogen ratio at least in the piliferous layer, and thus inhibit nodule formation. They have demonstrated that nodules are only formed if a soluble carbohydrate is also added to the external solution. If Lemmermann's hypothesis is accepted, it must be assumed that the absorption of inorganic nitrogen is fast enough to maintain an unfavourable carbon-nitrogen ratio in the piliferous layer, but not fast enough to provide the nitrogen necessary for maximum growth. To render this hypothesis tenable it seems necessary to make further assumptions. If the transference of nitrogen from the piliferous layer to the conducting tissues takes place at a slow rate, then sufficient nitrogen may not be translocated to the shoot unless the concentration of inorganic nitrogen in the root-hairs reaches a level much in excess of that required to prevent nodule formation. Again, one might put forward the view that in the absence of nodules, the transport of carbohydrates to the piliferous layer takes place at a slower rate, so that a lower level of nitrogen accumulation might inhibit nodule formation. Conditions under which sufficient nitrogen for growth can be obtained would seem to entail a high concentration of nitrogen, both in the external solution, and in the piliferous layer.

Evidence that the uptake of inorganic nitrogen is slow under conditions unfavourable for nitrogen fixation is available from unpublished work. It has been found that even when the amount of 'nitrogen fixed' is markedly reduced by lowering the light intensity, the addition of large quantities of inorganic nitrogen to the soil never leads to a large accumulation of nitrogen in the tissues. Grasses on the other hand under the same experimental conditions accumulate considerable amounts of nitrogen.

That the suppression of clover by nitrogenous manuring is associated with the reduction of nitrogen fixation is supported by the findings of a previous investigation (Blackman, 1934). It was there found that while an application of sucrose alone to the sward had no effect on the clover content, the addition of sucrose to either calcium nitrate or ammonium sulphate diminished very materially the rate at which the clover was reduced by nitrogen alone. On the basis of Allison's (1935), and Thornton's (1936) investigations it would be expected that the addition of sucrose would prevent the inorganic nitrogen from interfering with the formation of nodules. Furthermore, since the concentration of inorganic nitrogen in the soil was depressed to some extent by the application of sucrose, conditions more favourable to nodule formation would have been created.

The evidence so far cited suggests that the suppression of clover in the sward by nitrogenous manuring is brought about in two stages. Firstly, the high external level of inorganic nitrogen reduces nodule formation. Secondly, as a result of the absence of nodules insufficient nitrogen is absorbed for maximum growth. In regard to the second supposition, there are several difficulties. In the first place Thornton and Nicol (1934) have demonstrated that while the addition of sodium nitrate to lucerne (1-3 gm. per 20 kg. sand) had no adverse effect on growth, the same quantities of nitrogen applied to lucerne growing in association with *Lolium perenne* depressed its growth. In a later paper (1936) they also showed that such a concentration of nitrogen interfered with nodule formation when lucerne was grown alone. Moreover, Williams (1932), and Caldwell and Richardson (1936), found that ammonium nitrate and ammonium sulphate had no appreciable effect when applied to *T. repens*. In addition it will be demonstrated in a later paper that both ammonium sulphate and calcium nitrate applied under conditions very similar to these experiments did not depress the growth of clover. These findings, therefore, suggest that the depression of the clover by nitrogenous manuring is necessarily related to the presence of grasses.

It has been pointed out already that on the evidence of other experiments, *T. repens* does not absorb inorganic nitrogen as readily as do some grasses. When, therefore, inorganic nitrogen is added to clover growing alone, the external concentration of nitrogen will fall but slowly. Grasses on the other hand reduce the concentration very rapidly (Blackman, 1934). In the present series of experiments, the concentration of inorganic nitrogen in the soil would reach a peak immediately after each weekly application and then fall off

during the week. It is possible, that under such conditions of fluctuating concentration, nodule formation may not be initiated immediately the concentration falls from a high to a low level. If this is so then such fluctuations might still inhibit nodule formation, although the mean concentration might not. Moreover, with a sharply decreasing external concentration due to the presence of grasses, the internal concentration of nitrogen in the piliferous layer would not be maintained at a high level for a long period. As a result the amount of nitrogen reaching first the conducting tissue and finally the shoot might limit the growth of the aerial tissues.

There are however, two other ways in which the presence of grasses might interfere with nitrogen fixation. It has already been demonstrated (1934) that under conditions of these experiments the growth of the grasses is markedly increased by applications of nitrogen. This extra growth is likely to be associated with an increased output of carbon dioxide by the roots. This higher concentration of carbon dioxide might have an adverse effect on nodule formation. The smaller, therefore, the extra growth of the grasses produced by increased nitrogen supply, the less their effect in increasing the carbon-dioxide concentration. There remains the further possibility that the roots of the grasses besides carbon dioxide excrete some substance that inhibits nodule formation. Thornton (1929) has shown in the case of lucerne, that some substance which stimulates the nodule bacteria is excreted prior to nodule formation. It is possible that the grasses excrete an inhibitory substance in a similar way. The main objection to this hypothesis is that the rate of excretion must be bound up with active growth. It cannot be associated merely with increasing nitrogen supply, since under conditions in which growth is restricted by light intensity, additional nitrogen has little effect in suppressing the clover.

So far in this discussion the possible effects of additional nitrogen have been considered generally. The question now arises why ammonium sulphate depresses the clover more than calcium nitrate. Firstly, ammonia-nitrogen may be more effective than nitrate-nitrogen in the suppression of nodule formation and nitrogen fixation. Secondly, the inhibition of nodule formation may be due to the higher carbon-dioxide content of the soil due to increased respiration of the actively growing grasses. There is evidence both from past experiments (Blackman, 1934), and unpublished work that in full daylight under a system of constant cutting, ammonium sulphate increases the yield of a number of grasses more than calcium nitrate. Grasses therefore, growing in a sward manured with ammonium sulphate may compete more actively with the clover for nitrogen, and excrete more carbon dioxide. In addition, differences in the rate of growth brought about by ammonium sulphate, and calcium nitrate might differentially suppress the clover if competition was governed by other factors such as water, light, and nutrients other than nitrogen. Competition for water and light can be dismissed as unimportant. Except in 1933, the plots were frequently watered, while the herbage was cut

at such short intervals as to preclude any shading of the clover by the grasses. There remains, therefore, the possibility of competition for calcium, phosphorus and potassium. Competition for calcium seems unlikely since the experiments were carried out on neutral soils. In the case of ammonium sulphate the calcium carbonate, added in order to maintain the pH of the soil, at the same time supplied calcium in similar amounts to that added in the calcium nitrate. Competition for potassium and phosphorus again seems improbable. Firstly, numerous experiments on grassland at Jealott's Hill have shown that the application of potassic or phosphatic fertilizers, neither increases the yield nor encourages the development of clover. Secondly, the 1936 experiment, which showed the most striking differences between treatments was laid down on what had been a well established kitchen garden, with a very fertile soil. Thirdly, it has been demonstrated previously (Blackman, 1934), that at Jealott's Hill ammonium phosphate suppresses clover at the same rate as ammonium sulphate.

From the foregoing discussion it seems evident that the nature of the competition between grasses and clover will be dependent on the light intensity. It is clear that at all light levels, neither ammonium sulphate nor calcium nitrate has a direct effect on the clover except in so far as they affect nodule formation. The greatest depression of the clover occurs in daylight, where the response of the grasses to an increased nitrogen supply is largest. On the hypothesis advanced this suppression is likely to be associated with (i) competition for nitrogen resulting from a reduction of nitrogen fixation, and (ii) an increased carbon-dioxide content of the soil atmosphere due to respiration by the roots of the actively growing grasses. The superiority of ammonium sulphate over calcium nitrate in diminishing the clover may be attributed to (i) a greater depressing effect of ammonia-nitrogen on nodule formation, (ii) the more active growth of the grasses manured with ammonium sulphate, and in consequence a higher output of carbon dioxide, and a larger uptake of nitrogen. The comparative failure of additional nitrogen to suppress the clover under conditions of low light intensity may be attributed to the smaller response of the grasses to nitrogen. In fact it will be shown in a later paper that at light intensities of approximately 0.4 daylight the addition of ammonium sulphate, and more particularly calcium nitrate may actually depress growth.

Although this investigation has been primarily concerned with frequently defoliated swards, conclusions can be drawn as to the factors controlling the clover content under other sets of conditions. In these experiments the quantities of inorganic nitrogen added, and the consequent soil concentrations have been far in excess, either of those normally employed in agriculture or found occurring naturally in soils. It seems improbable that small quantities of nitrogen added at infrequent intervals will have if any but a transitory effect on nitrogen fixation. It is, therefore, not surprising that the clover content of a sward can be maintained in spite of normal nitrogenous manuring,

provided that it is frequently defoliated (Jones, 1933; Blackman, 1933). Infrequent defoliation on the other hand leads to the shading of the clover by the taller growing grasses, while the degree of shading will be dependent on their height and density. These experiments have shown that under conditions of frequent defoliation, which are favourable to the clover, a reduction in light intensity has an adverse effect on the clover content of the sward. Although it does not necessarily follow it seems probable that the light factor is still operative even when the clover is less frequently defoliated. Small quantities of nitrogen may therefore, indirectly suppress the clover since the nitrogen will increase the height and density of the grasses, and thereby increase the degree of shading.

Finally, for an adequate explanation of the results obtained in this investigation it has been necessary to put forward a number of assumptions, particularly assumptions concerning the process of nitrogen fixation. It is hoped in some cases to test these speculations by direct experimentation.

SUMMARY

The effects of variations both in light intensity, and nitrogen supply on the *Trifolium repens* content of *Agrostis* spp. dominant swards have been studied over a period of four years. Some eight experiments were carried out on four swards frequently defoliated in order to maintain conditions favourable to the clover. In each experiment additional nitrogen either as calcium nitrate or ammonium sulphate was added weekly at the rate of 37.5–50.0 lb. nitrogen per acre. Differences in light intensity were maintained by permanently shading the appropriate plots with butter muslin stretched one or more layers thick on wooden frames. Estimates of the clover content were made weekly, and the data analysed statistically by the method of covariance.

In seven out of eight experiments, the clover content fell markedly when the light intensity was reduced to 0.6–0.63 of daylight. In the five experiments where the light intensity was further reduced to 0.39–0.4 the content was decreased still more in only one experiment.

With decreasing light intensity the effects of additional nitrogen were less pronounced. Under the conditions of normal daylight, ammonium sulphate depressed the clover in four out of seven experiments, and calcium nitrate in three; in two of these experiments ammonium sulphate diminished the clover more than calcium nitrate. At the light level of 0.6–0.63, ammonium sulphate decreased the clover in three experiments and calcium nitrate in two. In none of the experiments were the effects of ammonium sulphate and calcium nitrate significantly different at this light intensity. At the lowest light level (0.39–0.4) ammonium sulphate brought about a reduction in three out of five experiments, and calcium nitrate in one. Ammonium sulphate was significantly different from calcium nitrate in two experiments.

On the basis of both these experiments and data as yet unpublished, it is concluded that the decrease in clover content brought about by shading is a

direct effect, and is not related to competition with the grasses. On the other hand the reduction in clover associated with additional nitrogen is primarily due to such competition. The intensity of this competition is linked with the active growth of the grasses, and is therefore greatest under conditions where light is not limiting their growth. The nature of this competition is obscure, it is suggested that competition for nitrogen plays a part, since with a high external concentration of inorganic nitrogen, nitrogen fixation will be at a minimum. It is concluded that the greater reduction in the clover content brought about by ammonia-nitrogen as against nitrate-nitrogen can in no way be associated with a direct toxic effect of absorbed ammonium ions. An alternative explanation is advanced.

In these experiments, very large quantities of inorganic nitrogen have been added to the sward. If small amounts are added nitrogen is likely to have little effect in determining the clover content. Where defoliation is infrequent, competition for light will predominate. Under these conditions small quantities of nitrogen may further depress the clover, since the increase both in height and in density of the grasses will lead to a greater shading of the more prostrate clover.

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Probable Causes of the Changes in Direction of the Major Spiral in *Trillium erectum* L.¹

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With Plates X to XII and one Figure in the Text

INTRODUCTION

IN studies where the major spiral of the chromonema in the first division of meiosis has been clearly seen it has generally been found that the direction of coiling may change somewhere throughout the length of the chromatid. Such changes are most common at the attachment (Sax (1935), on *Rhoeo discolor* and *Tradescantia*; Matsuura (1935), on *Trillium kamtschaticum*; Huskins and Smith (1935), on *Trillium erectum*). Sax and Humphrey (1934), and Nebel and Ruttle (1936), have found some interstitial changes in *Tradescantia*. Taylor (1931) illustrated several, without comment, in *Aloe*. Sax found that in *Rhoeo* such changes are rare. *Trillium erectum* and *Trillium kamtschaticum* apparently may have numerous changes in direction of coiling occurring at any point along the length of the chromatid. Huskins and Smith, Sax and Humphrey, and Nebel and Ruttle suggest that these interstitial changes may be due to chiasmata. Huskins and Smith make this suggestion on the grounds that there are roughly twice as many changes as chiasmata, if those at the attachment are excluded. They point out, however, that reconstructions of the chiasmata from anaphase chromosomes do not explain all the observed changes.

There are three obvious factors which could cause changes in direction of the major coil: (1) chiasmata, (2) the attachment, and (3) random changes which may be expected to be proportional to the number of gyres which the chromonema forms. It has been shown (Huskins, Wilson, *et al.*, 1937) from comparisons of normal synaptic, desynaptic, and asynaptic *Trillium* that all three factors play a part. The detailed data are here presented.

MATERIALS AND METHODS

Many of the data on normal *Trillium* are from Huskins and Smith, and direct measurements of chromosome lengths were made from the slides on

¹ These and other related studies were made possible by the generosity of the Rockefeller Foundation in providing funds for controlled temperature chambers and other equipment.

which their paper was based. Additional data on the normal chromosomes were supplied by Dr. A. W. S. Hunter (1937), who worked in part on the same material as Huskins and Smith. Dr. Hunter also contributed the data on the asynaptic material.

The studies of the desynaptic material were made on a plant which had gone through the post-diplotene stages at a temperature of 9° C. This plant was one of a series which were exposed to various temperatures from 0° C. to 36° C. in an attempt to determine the effects on meiosis. That it was actually desynaptic and not asynaptic is shown by the fact that in most cells some chiasmata were not completely resolved and in others the positions of the chromatids indicate closer association in earlier stages. That it was not normal is shown by the fact that even as early as metaphase sister chromatids were separated.

Staining was according to the standard iodine crystal-violet procedure after fixation in La Cour's 2BD. This procedure was also used in the normal preparations of Huskins and Smith and of Hunter. The asynaptic studies were made on aceto-carmin preparations.

Observations were made with a Zeiss 120×, 1.3 N.A., and a Zeiss 90×, 1.3 N.A. objective combined with 15× and 7× oculars.

DATA

Normal synaptic material.

The chromonemata of the anaphase chromosomes of *Trillium erectum* shown in Pl. II, Fig. 18, of Huskins and Smith (1935) have forty-five changes in direction per cell. The chiasma frequency of this material is given as 17.15 per cell at late diakinesis, but the authors point out that the individual chromonemata were not followed through the chiasmata in obtaining this figure. The number of half gyres per chromatid has been calculated from Huskins and Smith's figures, and it has been found that the mean number per five chromatids (one each of chromosomes A, B, C, D, and E) is 116. There is practically no variation between chromatids of the same chromosome.

Hunter has contributed similar data which agree very well with those given above. He reports forty-seven changes in direction for one cell, of which the entire complement was analysed at anaphase. He gives 15.8 as the mean chiasma frequency at metaphase of this material in which chromatids were followed individually through chiasmata. The total number of half gyres for five chromatids has been determined in this cell and found to be 118.

Further calculations were made on twenty-six isolated chromosomes drawn by Hunter. The figures obtained from these calculations were 115.9 half gyres per set of five chromatids and 45.8 changes in direction per set of five bivalent chromosomes.

'Chromosome lengths' (as distinct from chromonema lengths) were calculated from the illustrations in Huskins and Smith's publication and were

also measured directly from the original slides with a micrometer. The total length per five chromosomes was found to be about 61.5μ by calculation from the twelve anaphase chromosomes shown in Huskins and Smith's Plate II. Direct measurements of twenty-seven chromosomes gave a mean length per five chromosomes of 60.0μ with practically no deviation from the mean.

Desynaptic material.

Complete sets of chromosomes were very clear in two cells of this material (Pls. X, XI, XII, Figs. 1-7), so that all twenty chromatids in each cell could be drawn with considerable accuracy. One of these cells had thirty-three changes in direction and the other had thirty-four.

Counts of the changes in direction in chromatids of a number of other cells in which the entire complement could not be drawn accurately indicated that the two complete cells studied were typical and that the consistency of the results was not due to coincidence.

The first cell had an average of sixty-nine half gyres per five chromatids, while the second had sixty-six.

The total mean length of the five chromosomes measured directly was 36.3μ . The diameter of the gyres was the same as the synaptic, i.e. about 1.5μ .

No significant figures on the chiasma frequency could be obtained since in all cells the chiasmata were at least partially resolved at stages obviously well before anaphase.

Asynaptic material.

Hunter reports two types of asynaptic cells in his material, one having no association between homologous chromosomes (type 1) and the other having no association between either homologous chromosomes or sister chromatids (type 2).

No cells of type 1 were found in which all the chromatids could be studied accurately, but calculations on a number of chromosomes from different cells gave an average of thirty changes in direction per cell and 144 half gyres per set of five chromatids.

One cell of type 2 was found in which the complete chromatid complement could be studied. In this there were forty-two changes in direction and 209 half gyres per five chromatids.

Since these studies were made on aceto-carmin preparations, chromosome measurements are not comparable to those of the synaptic and desynaptic material. There is, however, good evidence that the number of gyres may be used as a substitute for length; the ratio of the number of half gyres to the total length of five chromatids is the same for the synaptic and desynaptic materials (Table II) and the same relation holds for the two types of asynaptic material (Table III).

A summary of the data from all types of material studied is given in Table I.

TABLE I
Number of Half Gyres, Chromatid Lengths, Chiasma Frequencies and Number of Changes in Direction in Synaptic, Desynaptic, and Asynaptic Trillum

Chromosome.	Synaptic.		Length of chromatids.	Desynaptic.		Asynaptic.				
	Half gyres. (Huskins and Smith.)	(Hunter.)		Half gyres. Cell. 1.	Cell. 2.	Length (mean of 1 and 2).	Half gyres Type 1.	Length Type 1.	Half gyres Type 2.	Length Type 2.
A	21	20	8.5	12	12	5.6	22	12.2	35	17.2
B	24	24	10.0	11	12	6.3	31	12.7	40	18.1
C	18	18	10.5	12	11	6.3	23	12.6	32	16.3
D	23	24	11.5	12	12	7.5	28	15.9	42	19.0
E	30	32	19.5	19	20	10.6	40	20.4	60	29.0
Total	116	118	60.0	66	67	36.3	144	73.8	209	99.6
Chiasma frequency	17.15 (Huskins and Smith) (diakinesis; matrix stained)			Indeterminable			None		None	
	15.8 (Hunter) (metaphase; chromonemata only stained)									
Changes in direction	45 (Huskins and Smith) 47 (Hunter)			33 (cell 1) 34 (cell 2)					30 (type 1) 42 (type 2)	

TABLE II

*Relation between Number of Half Gyres and Chromosome Length,
2 BD Fixation*

Material.	No. half gyres (five chromatids).	Chromosome length (five chromatids).	No. half gyres Length.
Synaptic	116.5	60.0	1.8
Desynaptic	66.5	36.3	1.8

TABLE III

*Relation between Number of Half Gyres and Chromosome Length, Aceto-
carmine Material*

Material.	No. half gyres (five chromatids)	Chromosome length (five chromatids)	No. half gyres Length
Asynaptic { Type 1	144	73.8	1.9
Type 2	209	99.6	2.0

TABLE IV

*Relation expected between Number of Chiasmata and Changes of Direction
of Coiling, on Various Assumptions*

1a. Direction of coiling random and changes optional at chiasmata	Chiasmata : changes = 1 : 1
b. Direction of coiling random and changes obligatory at chiasmata	Chiasmata : changes = 1 : 2
2a. Direction of coiling opposite and changes optional at chiasmata	Chiasmata : changes = 1 : 1
b. Direction of coiling opposite and changes obligatory at chiasmata	Chiasmata : changes = 1 : 2
3a. Direction of coiling the same and changes optional at chiasmata	Chiasmata : changes = 1 : 1
b. Direction of coiling the same and changes obligatory at chiasmata	Chiasmata : changes = 1 : 2

DISCUSSION

Huskins and Smith (1935) observe that the number of changes in direction (omitting those associated with the attachment) are approximately equal to twice the chiasma frequency, which might suggest that chiasmata are the causes of such changes. On this assumption they have reconstructed metaphase figures from the anaphase chromosomes in an effort to find out whether or not all interstitial changes in direction could be explained as being due to chiasmata. They found that a certain number of changes definitely did not fit this hypothesis, and these must have some other explanation. It, nevertheless, seems probable that chiasmata do in some measure affect the number of changes in direction. Since coiling begins after chiasma formation (Huskins and Smith) chiasmata may be looked upon as points of interruption in the coiling and would be expected to affect the direction of coiling in one of six ways. The direction of coiling in the two chromatids involved in a chiasma

(homologues on the partial chiasma-type hypothesis) might in any given segment be (1) random, (2) opposite, (3) the same. In each of these cases changes in direction of coiling might be (a) optional, (b) obligatory.

Alternatives 1*a*, 2*a*, and 3*a* all give 1 : 1 ratio of chiasma frequency to number of changes in direction while 1*b*, 2*b*, and 3*b* give a 1 : 2 ratio (see Table IV). It follows that postulates number 1*b*, 2*b*, and 3*b* all account fairly well numerically for the interstitial changes in direction, and this number added to approximately ten changes associated with the attachments (since there is evidence that these changes are random) gives an expectation of forty-two to forty-four changes as compared with forty-six and forty-seven observed. Two of these possibilities (2*b* and 3*b*) are definitely ruled out by observation since we have found on detailed analysis, contrary to a previous (1935) general impression, that the direction of coiling is not predominantly opposite (or the same) in paired segments. For the same reason alternatives 2*a* and 3*a* must also be rejected. This leaves only alternatives 1*a* and 1*b* to consider in detail. The latter, as pointed out above, gives a good numerical fit but as a working hypothesis has two serious faults: (1) many observed changes in direction in anaphase chromonemata cannot be explained by any reconstruction of antecedent metaphase chiasmata (Huskins and Smith, 1935); (2) asynaptic material, which never had chiasmata, has a relatively large number of changes.

On alternative 1*a* a chiasma frequency of seventeen would be associated with seventeen changes in direction. This added to an assumed number of ten changes associated with the attachments would give a total of twenty-seven changes compared to forty-six and forty-seven observed, leaving twenty to be accounted for in some other way.

Table V shows the number of changes in direction observed in the three types of material studied and the number expected on alternatives 1*a* and 1*b*.

If most of the changes in direction were due to chiasmata then we should expect only about ten changes per cell in the asynaptic material, whereas there are three to four times this many.

The fact that there are more changes in direction in the longer asynaptic chromosomes (type 2) than in the shorter (type 1) leads one to suspect that the number of changes (with the exception of those associated with the attachments) may vary with the length of the chromonema or with the number of gyres. If this is so, then the fact that in type 1 asynaptic there are about 144 half gyres (per set of five chromatids) associated with twenty changes in direction (omitting ten assumed to be due to the relative independence of chromosome arms on either side of the attachment) then 116 half gyres in normal synaptic material should be associated with seventeen changes. This is much lower than the number observed, but it will be noted (Table VI) that if we add this number to that assumed to be associated with the attachments and to the number expected on alternative 1*a* we get a total of forty-four changes which agrees very well with the observed frequency.

From the above results it is deduced that three factors are involved in the causation of changes of direction in the major coil of the meiotic chromonema in *Trillium erectum*: (1) chiasmata which cause a number of changes per cell

TABLE V
Number of Changes in Direction per Cell Observed and Expected on Alternatives 1a and b

Material	Chiasma frequency	No. of changes expected on alternative 1a (xta + attach.)	No. of changes expected on alternative 1b (xta + attach.)	No. of changes observed.
Synaptic:				
H. and S.	17·15	27 (17 + 10)	44 (34 + 10)	45
Hunter	15·8	25·26 (15, 16 + 10)	40·42 (30, 32 + 10)	47
Desynaptic:				
Cell No. 1	indeter-	—	—	33
Cell No. 2	minable	—	—	34
Asynaptic				
Type 1	o	10 (attach.)	10 (attach.)	30
Type 2	o	10 (attach.)	10 (attach.)	42

TABLE VI
The Number of Changes in Direction assumed to be associated with Chiasmata, the Attachments and the Number of Gyres

	No. of changes
Expected from alternative 1a (normal material)	17
Expected to be associated with the attachments	10
Expected to be proportional to the number of gyres	17
Total	44
Observed in normal material	45 and 47

approximately equal to the chiasma frequency; (2) the attachment which allows the direction of coiling to be random in chromosome arms thus giving a number of changes about equal to half the number of chromatid attachments (Hunter on *Trillium* and Nebel and Ruttle on *Tradescantia*); and (3) the length of the chromonema and chromosome which are correlated with the remaining number of changes. This latter factor will be analysed further in a subsequent communication.¹

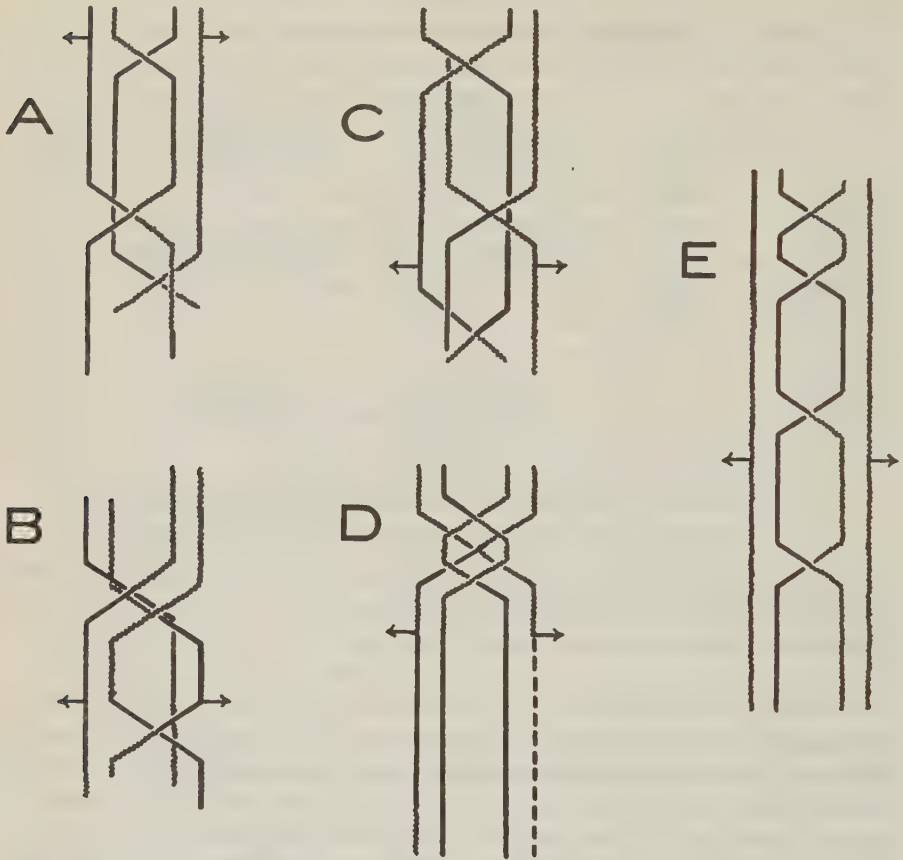
The changes in direction observed in the asynaptic material also fit in with this hypothesis, but in this case the chiasma factor is, of course, eliminated.

The desynaptic material provides a further test of this hypothesis. It will be noted (Table I) that the number of half gyres per five chromatids is very much lower in it than in either the normal or asynaptic, and that the number of changes is also lower than normal and lower even than the second asynaptic type which has particularly long chromonemata. On the hypothesis being

¹ Dr. M. J. D. White has recently shown us changes of direction elsewhere than at the attachment in mitotic (spermatogonial) chromosomes of a grasshopper; obviously these cannot be due to chiasmata.

considered there are three possible explanations for this fact: (1) A departure from randomness in the number of changes associated with the attachments; (2) a lower chiasma frequency than normal; and (3) the smaller number of gyres.

From direct observation it seems that there is no great departure from



Diagrams illustrating reconstruction of possible chiasmata of the five bivalents shown in Pl. I, Fig. 1, assuming that sister chromatids are always associated at their attachments; — dextorse coiling, — sinistrorse coiling.

randomness in the number of changes at the attachments. The chiasma frequency was indeterminable in this material as there were no stages early enough to show chiasmata before the chromosomes had at least partially separated. A number of cells were found (Pl. XII photomicrographs 8-11), however, in which chiasmata were still present, but never more than one or two per bivalent, and in most cases these were on the point of being resolved by passing off the ends. In one cell (photomicrograph 11) there were five or six such chiasmata. We may therefore assume that there was a chiasma frequency of not less than five per cell. Since bivalents with two chiasmata

were often encountered we must assume that the mean chiasma frequency was greater than five, but no upper limit can be set.

The text-figure is a diagrammatic reconstruction of the five bivalents so made that chiasmata might explain as many changes as possible. The only restriction adopted in this reconstruction was that sister chromatids were assumed to be associated at their attachments. It will be seen here that twenty changes could be caused by the sixteen chiasmata which can be assumed on this basis.

TABLE VII

The Ratios of the Number of Changes in Direction per Cell to the Number of Half Gyres per Five Chromatids

Material.	Total changes. No. half gyres.	Assumed no. of changes due to xta. and attach.	Remaining changes.	Remaining changes. No. half gyres.
Synaptic	0.39	27 (17+10)	20	0.17
Desynaptic	0.50	27 (17+10)	7	0.10
Asynaptic:				
Type 1	0.21	10 (attach)	20	0.13
Type 2	0.20	10 (attach)	32	0.15

This agrees much better with the 1:1 expectation than with the 1:2 alternative.

On the assumption that a certain number of changes are associated with the number of gyres and that all (with the exception of those associated with the attachments) in the asynaptic chromosomes are of this type, then the sixty-six half gyres of the desynaptic type should be associated with about nine changes. This would leave about twenty-four changes, of which ten would occur at the attachments and fourteen would have to be accounted for by chiasmata. The mean chiasma frequency of normal material was found to be sixteen to seventeen, and it is possibly somewhat lower in the desynaptic material.

It therefore appears that the desynaptic material also fits this hypothesis.

If the hypothesis is correct it must satisfy the following tests. On it we can divide the causes of changes in direction into two types; one type (associated with chiasmata and the attachments) being relatively stable with reasonable variations relative to chromosome length, and the other varying markedly and directly with the number of gyres. If this is true then the ratio of total changes to the number of half gyres per set of five chromatids should be much greater in the shorter desynaptic chromosomes than in the synaptic since the relatively stable type of cause is more significant in the former. Also, if we eliminate those changes due to the relatively stable type of causes, the ratio of the remaining changes to the number of half gyres per five chromatids should be the same in synaptic, desynaptic and asynaptic material. These calculations are presented in Table VII. The assumptions made in constructing this table were: (1) that in each type of material ten changes per cell were

associated with the attachments and (2) that the chiasma frequency of the desynaptic was the same as that of the normal synaptic. If the second assumption is slightly in error, as is probable, and the chiasma frequency of the desynaptic material *at the time of initiation of coiling* was slightly lower than normal then the ratios for all types of material would be in relatively close agreement.

CONCLUSIONS AND SUMMARY

From the foregoing analysis of the data gathered from synaptic, desynaptic, and two types of asynaptic *Trillium* it would seem that changes in direction of the major coil are associated not only with chiasmata and the attachments, but that their frequency also varies with the number of gyres per chromatid. An hypothesis which appears to fit the data is formulated as follows: (1) Chiasmata may cause a number of changes equal to the chiasma frequency. (2) The direction of coiling is random on either side of the attachment; this will cause half as many changes in direction as there are chromatid attachments. (3) The remaining number of changes is proportional to the number of gyres. These changes must be due to some factor associated with the gyre frequency. The possible nature of this factor will be considered in a subsequent paper.

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EXPLANATION OF PLATES X TO XII

Illustrating Professor C. L. Huskins and Mr. G. B. Wilson's paper on 'Probable Causes of the Changes in Direction of the Major Spiral in *Trillium erectum* L.'

PLATE X

Magnification 5,000

Fig. 1 (A-E). Complete complement of a desynaptic cell drawn for analysis of changes in direction. The 'tertiary' split is shown in several chromatids.

PLATE XI

Magnification 5,000

Fig. 2 (A-E). Complete complement of a second desynaptic cell.

Fig. 3. A reconstruction of the cell analysed in Fig. 1. Cf. photomicrograph, Fig. 4.

PLATE XII

Photomicrographs. Magnification about 2,500

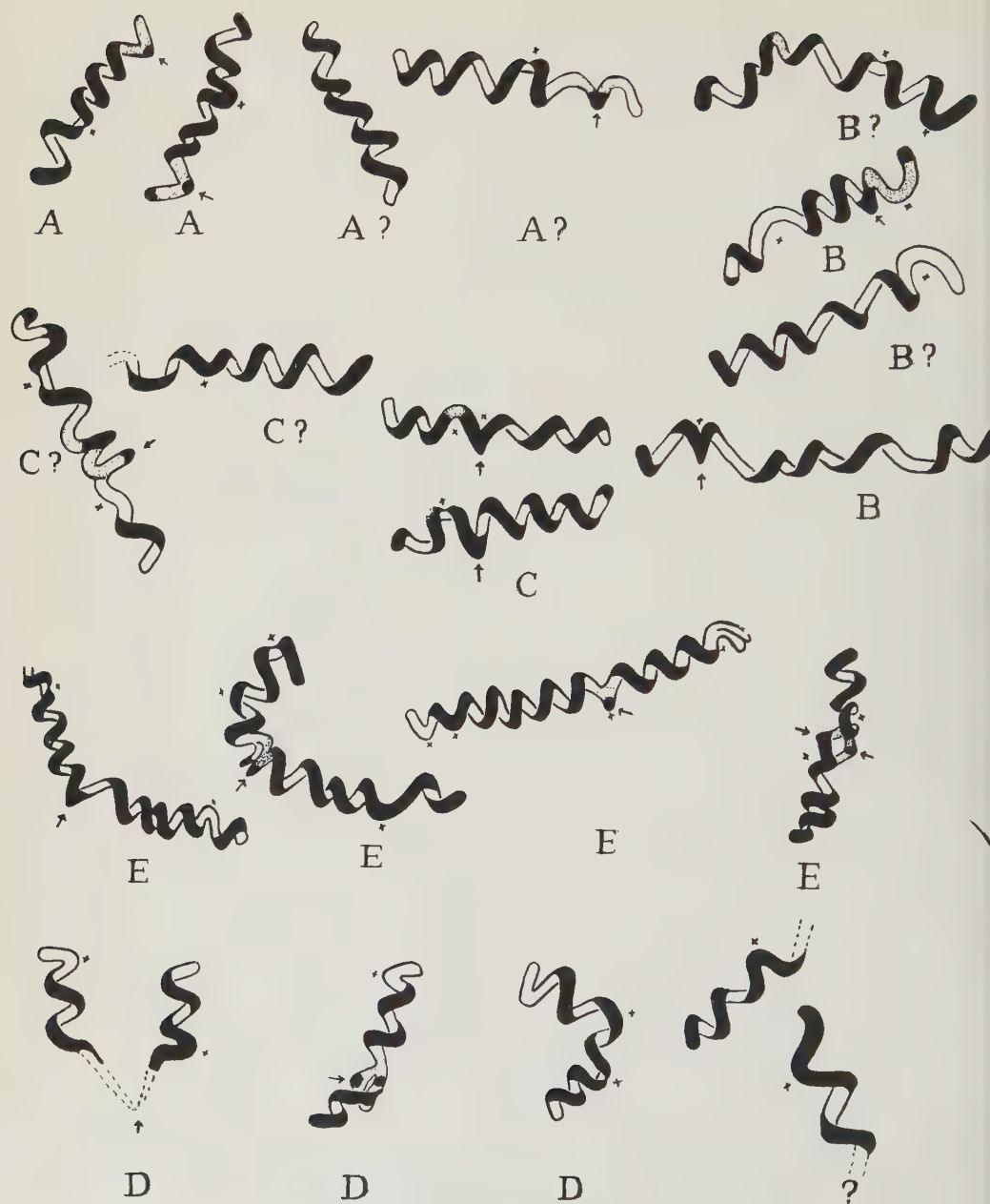
Fig. 4. The desynaptic cell analysed in Fig. 1. Probably early anaphase.

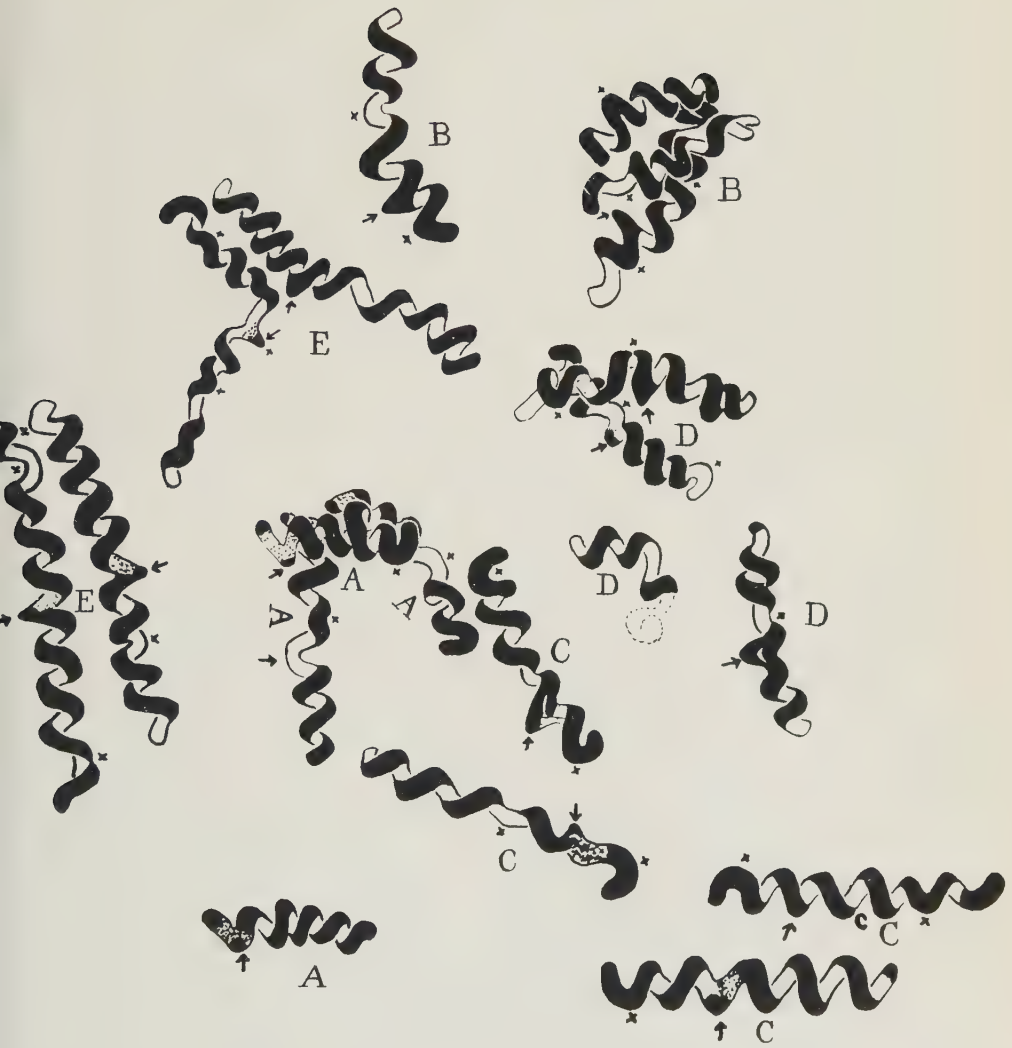
Figs. 5, 6, and 7. Three levels of the cell drawn in Fig. 2.

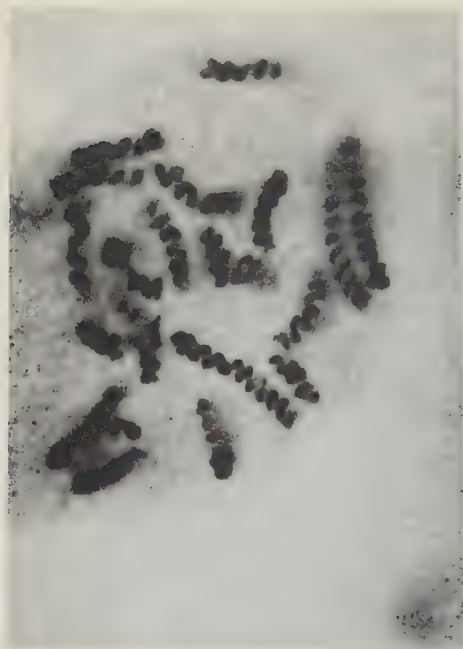
Figs. 8, 9, and 10. Three cells showing chiasmata on the point of being resolved.

Fig. 11. A cell showing chiasmata on the point of being resolved in each of the five bivalents.

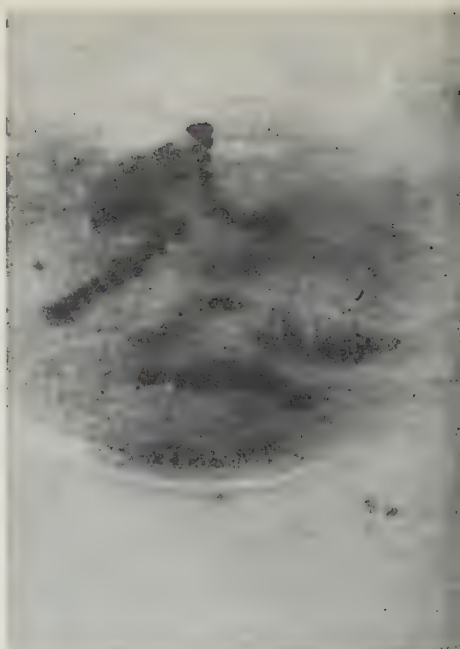




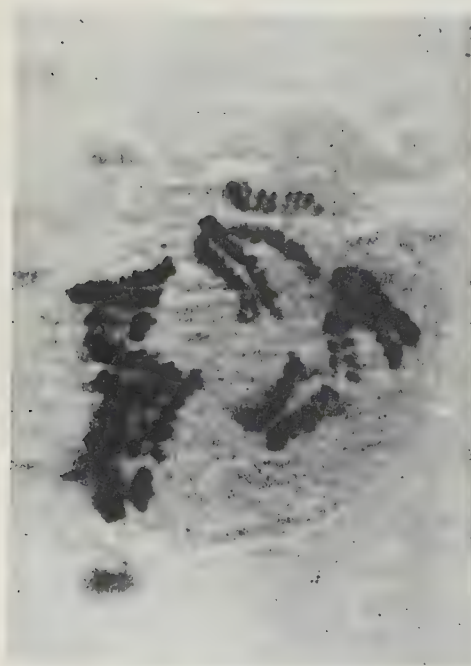




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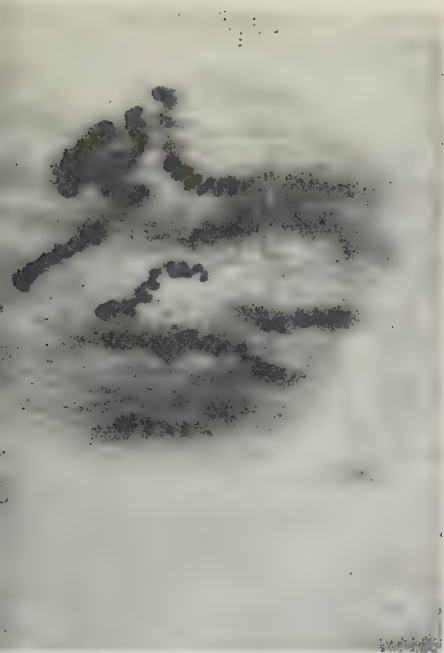
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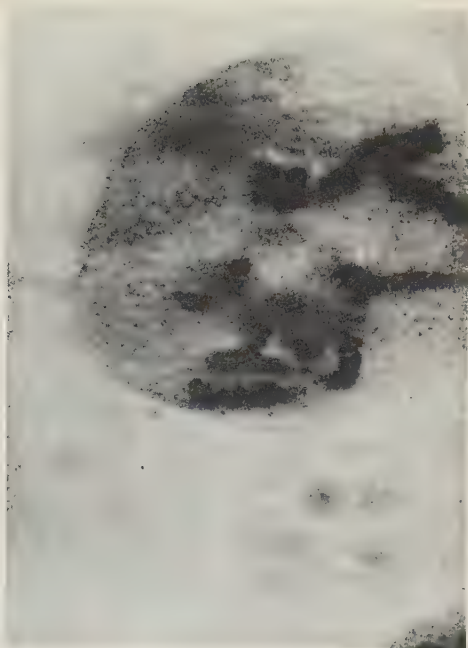
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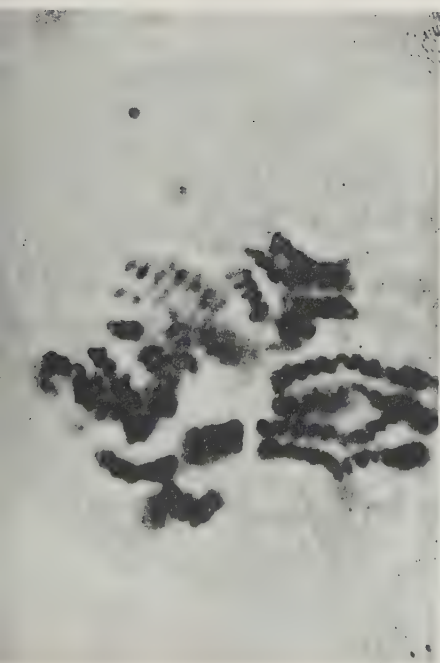
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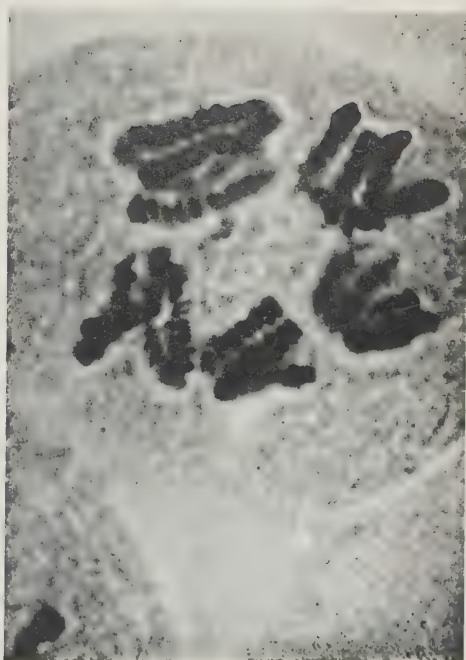
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Huth, Stubbs X., Kent

Possible Action of Phytohormones as Root-determiners

BY

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With twelve Figures in the Text

I. INTRODUCTION

THERE are obviously two possible explanations of the fact that the meristems produced by treating the stems of plants with phytohormones develop into roots and not shoots. The first is that the phytohormones also act as 'root-determiners', i.e. they cause the meristem to develop into a root rather than a shoot. The second is that the meristems so induced, being formed from the cambial regions, are internal and not external, the normal origin of shoots being exogenous and of roots endogenous. Thus Tincker (1937) has suggested that the pressure of the tissues surrounding these new meristems might mould the parenchyma into cylindrical roots.

The following experiments and observations suggest that the phytohormones act as root-determiners. In fact, when the formation of meristems has been stimulated by a dose of hormone, adventitious shoots instead of roots may be formed in the absence of a further supply. Normal axillary buds, or parts of them, have also been observed to grow into roots when hormone was applied to the stem.

2. EXPERIMENTAL TECHNIQUE

It has been shown in various investigations that phytohormones are manufactured in developing buds and leaves, e.g. Went (1929), Thiman and Skoog (1934), and Avery (1935). From these situations they move morphologically downwards. Thus, for example, root development is stimulated at the base of a cutting. In most of the experiments here to be described decapitated plants, as shown in Fig. 1, were used. These plants have a region of stem higher than buds or leaves.

The investigation to be described was part of an attempt to obtain tetraploid Brassicæ by somatic doubling and so the plants used were seedlings of kale, *Brassica oleracea*. They were decapitated when they had two to four large leaves. At the same time the axillary buds were removed, but this operation was not always complete and axillary buds were then removed at later

horseshoe-shaped reservoir made of plaster of paris cemented to a sheet of glass. The opening in the plaster was sufficient to allow of the stem passing through it and was rendered watertight with vaseline. The stem lay horizontally on the glass plate under the microscope and a suitable bubble being found its length was measured at short intervals by means of an eyepiece micrometer.

When the leaves were retained on the piece of stem examined it was found that most bubbles moved more or less rapidly up the vessels. This rendered

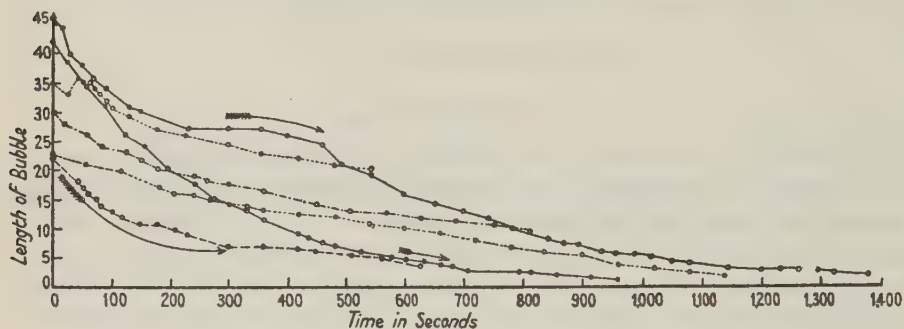


FIG. 1.

measurement difficult and sometimes impossible, so the procedure was modified so that after removing the epidermis from one side of an internode the latter was cut out from the rest of the plant. The basal surface was then dried and a bubble of air drawn in by sucking the apical end; the internode being immediately placed on the glass plate as before. In the figures only the three curves of Fig. 1 which are shown with arrows attached are of bubbles in stems with the leaves attached, the others being in stem internodes only.

Stems killed with chloroform were immersed in chloroform-saturated water overnight and then washed for some hours in tap-water. Those killed in boiling water were boiled for an hour and then either immersed in air-saturated water overnight or placed directly in a bottle with the water in which they were boiled. The bottle was immediately corked and cooled rapidly to room temperature. Stems killed by boiling were so soft that it was found more convenient to crush the stem between two glass plates after the air bubble had been drawn in than to mount it as described for other material. This method also had the advantage that the plates served to exclude air when it was desired to keep the tissues in air-free water.

Semipermeable collodion tubes were prepared by immersing pieces of 34 s.w.g. bore copper wire in 'collodium flexile', withdrawing them and allowing the collodion to dry. The wires were then immersed in 75 per cent. alcohol to render the collodion coating permeable, after which they were placed in dilute nitric acid solution until the wire was dissolved, when the tubes were rinsed several times in water. Air was admitted to the tubes so formed by laying short lengths of tube on a slide and allowing them to dry

slowly, when a bubble of air would enter at one or other end. The tube was then immediately immersed in water and the size of the bubble measured as already described.

Where bubbles of oxygen or nitrogen were used in place of air, scraped internodes were cut out under water and the basal end of each pushed into a

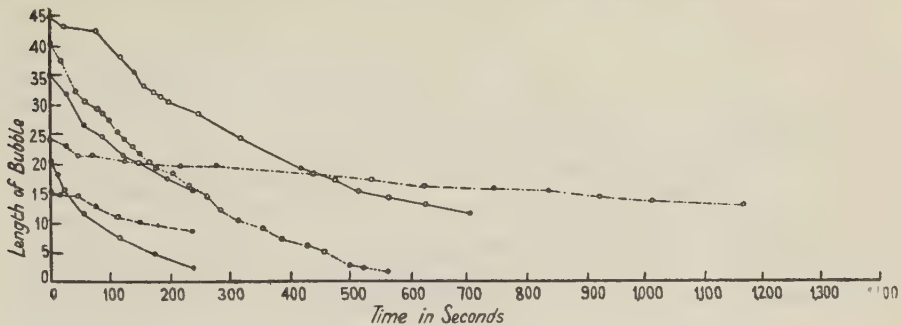


FIG. 2.

tube delivering the required gas. As it was not possible in this case to dry the cut surface it was found necessary to allow the gas-jet to impinge on the surface for some time to ensure the entry of bubbles of the gas into the vessels.

3. RESULTS AND DISCUSSION

The rate of disappearance of gas bubbles was determined under eight different conditions, of which six are shown in the figures.

Fig. 1 shows the change in size of air bubbles in tissue which had been kept in the light for twelve hours and which was illuminated during the experiment, i.e. there was a lamp focused on the internode in its position under the microscope and the microscope lamp was left on continuously. As already stated, the three curves with arrows were of bubbles in a stem to which the leaves and apex were attached. The tail of the arrow shows the position when the bubble began to move and the head marks the time when it became stationary again.¹ Fig. 2 is of air bubbles in material kept in the dark before and during the experiment, the microscope lamp being turned on only for the time required to take a reading. Figs. 3 and 4 are of air bubbles in stems killed in boiling water, and examined in air-saturated and in air-free water respectively. Fig. 5 shows air-bubble size in chloroform-killed tissue examined in air-saturated water. Fig. 6 is of two air bubbles in collodion tubes. In the figures

¹ It is of interest to note that in two of the three cases illustrated in Fig. 1 where the bubble moved during the time readings were being taken (in the third case movement only occurred for a very short time), the decrease in the length of the bubble was temporarily arrested, though subsequently the rate increased, with the result in each case that the final size of the bubble was much the same as that to be expected had no movement occurred. What bearing this may have on the question of the mechanism of absorption is not clear, but it indicates that a temporary small tension in the water column has no ultimate effect on the disappearance of an air bubble.

each line represents the sizes at different times of a single bubble, and all the figures with the exception of Fig. 6 are drawn to the same scale. The majority of the vessels in which bubbles were examined were of the order of $17\text{--}30\ \mu$

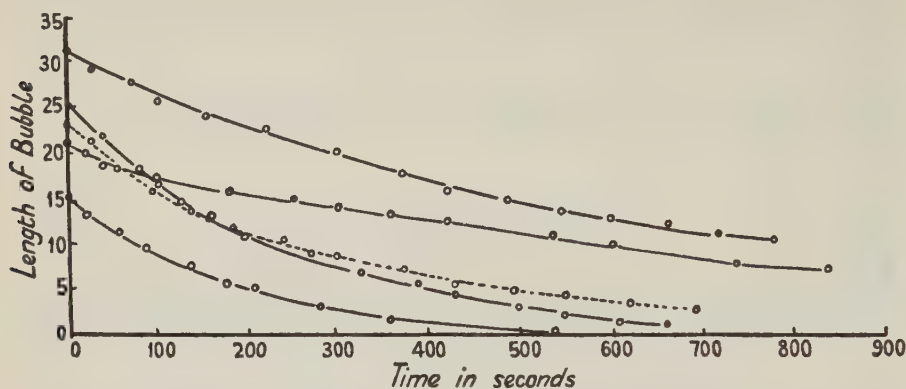


FIG. 3.

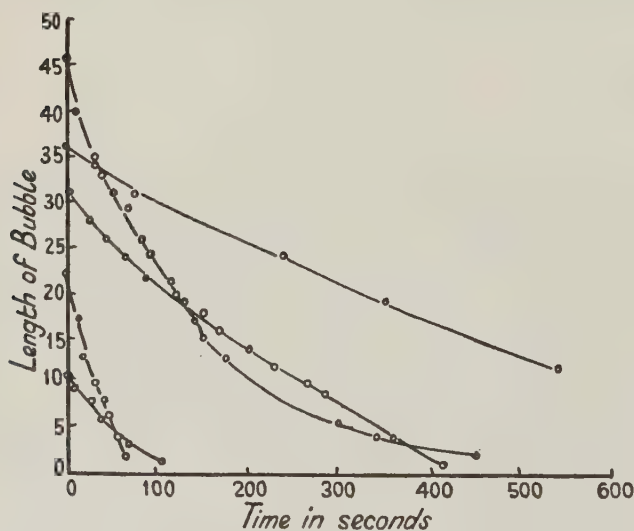


FIG. 4.

diameter with the exception of those in Fig. 4 which were some $60\text{--}80\ \mu$ wide. Each unit of length represented on the graphs equals $17\ \mu$.

Curves showing bubbles of nitrogen and oxygen were also obtained. These were similar to one another and to those for air and so are not shown. The fact that oxygen (which is twice as soluble as nitrogen), nitrogen, and air all give somewhat similar curves indicates that difference in the solubility of the gases is relatively an unimportant factor in the rate of absorption of the bubbles.

An examination of the graphs shows that there is considerable variation in the rates of disappearance of the different bubbles, and this makes difficult a

comparison of the effects of the various treatments. The curves in all cases are of a similar type in that there is at first a rapid decrease in length, i.e. size, which becomes progressively slower as the bubble gets smaller. This is of course to be expected, as the area of absorption is proportional to the length

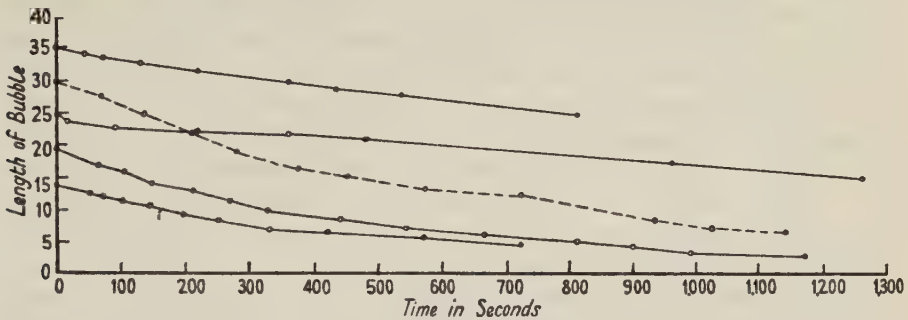


FIG. 5.

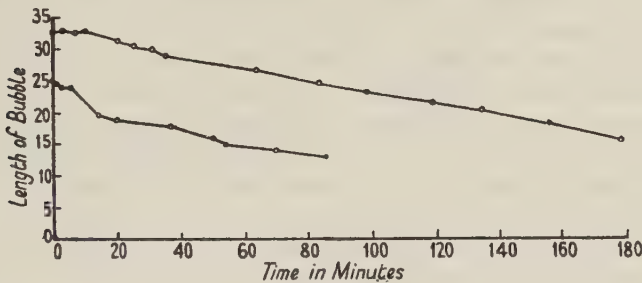


FIG. 6.

of the bubble, assuming a constant diameter for the vessel. A comparison of Figs. 1 and 2, in which the treatments differed in that the tissues were exposed to light in Fig. 1, and kept as far as possible in the dark in Fig. 2, shows that on the whole bubbles are absorbed more slowly in illuminated tissue than in material kept in darkness. Such a difference is in support of the hypothesis put forward in the first paragraph. In tissues killed by boiling or immersion in chloroform water—Figs. 3 and 5 respectively—absorption of bubbles is generally as slow (or slower in the latter case) as it is in illuminated living material. This indicates in turn that the theory outlined, while it may well be a factor, is not the only or the most important factor influencing bubble absorption. It may be the case, however, that in dead tissue the lack of protoplasmic streaming which would act in removing gas dissolved from the bubbles, and thereby increase the concentration gradient and with it the rate of solution of the gas, is accountable for the slower rate of absorption of the bubbles.

That absorption of the gases of bubbles occurs in the absence of living tissue is evident, as it is found not only in dead plant material but in semi-permeable collodion tubes, as shown in Fig. 6. The comparative slowness of

solution of gas in the collodion tubes used is to be expected since their internal diameter of some 178μ is from 5 to 10 times that of the xylem vessels. The rapid absorption of gas seen in Fig. 4 is to be expected owing to the steep diffusion gradient into air-free water.

It is concluded that the disappearance of air bubbles from the vessels and tracheides which has been observed under experimental conditions is due mainly to the increased pressure on the gases of the bubbles which results from the surface tension forces acting on these small bubbles, the rate of solution being increased as a result of their elongated form. No evidence was obtained for the view that increase in the partial pressure of the nitrogen in the bubbles, which would result from the absorption of oxygen by the surrounding living tissues, plays any part, though on *a priori* grounds some effect of this kind would seem inevitable.

It is of interest to restate here the conclusions arrived at by Sen and Blackman (1933) concerning the somewhat analogous case of the replacement by water of the air contained in intercellular spaces of the leaves of land plants submerged in water. They showed that injection of the intercellular spaces occurred in darkness but not in the light, and concluded that as the oxygen of the air in the intercellular spaces is used up in respiration and the carbon dioxide produced is mostly dissolved in the water, the gas pressure falls and water is drawn through the stomata causing partial injection of the leaf spaces. At the same time the removal of oxygen results in an increase in the nitrogen concentration above that in the air so that the partial pressure of nitrogen is greater than that with which the surrounding water is in equilibrium. This causes the nitrogen to go into solution and complete injection of the spaces occurs.

It is recognized that the bubbles considered in the present paper are experimentally introduced into the xylem and *exposed neither to compression by root pressure nor to any considerable tension from the transpiration 'pull'*. Bubbles in the xylem appearing in the intact plant—if they arise at all—would result presumably from a breakage of the water columns. The space, i.e. bubbles, thus produced would at first contain only water vapour into which oxygen and nitrogen would diffuse until equilibrium with the gases dissolved in the surrounding fluid is reached. While the conditions remain the same these bubbles of course will not redissolve.

The experiments described in this paper have a direct bearing on the capacity for recovery of cut flower shoots which have been left without a water supply for considerable periods. Such shoots should have all their water-conducting elements blocked with air bubbles so that when immersed in water there should be little absorption from the cut surface. That cut shoots under these conditions do, however, often regain their power of water absorption and conduction is a matter of common observation. The explanation is now obvious, the bubbles of air disappear for the same physical causes as do those of the *Impatiens* shoots experimented with.

4. SUMMARY

Bubbles of air, nitrogen, and oxygen introduced into the xylem vessels of both living and dead tissues of *Impatiens parviflora* were followed under the microscope and in all cases were found to decrease in size until completely dissolved in the cell-sap of the surrounding tissues. There was considerable variation in the rate of their absorption, the time required for the disappearance of an average-sized bubble ranging from twelve to twenty minutes. Similar results were obtained with bubbles contained in semipermeable collodion tubes, though in this case the rate of absorption was much lower, due, at least in part, to the diameter of the collodion tubes being much larger than that of the xylem vessels.

It is concluded that the disappearance from the tracheides and vessels of these artificially-introduced air bubbles is due mainly to the increased partial pressures of the gases of the bubbles resulting from the high surface-tension forces acting on the small bubbles. No evidence was obtained for the view that increase in the partial pressure of nitrogen in the bubbles, resulting from the absorption of oxygen by the surrounding living tissues, plays any part, though some such effect would seem inevitable.

The bearing of the results obtained on the ability of cut flower shoots to obtain supplies of water even after the cut surfaces of their stems have been exposed to air is discussed.

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Studies in Tropical Fruits

III. Preliminary Observations on Pneumatic Pressures in Fruits

BY

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With eight Figures in the Text

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I. INTRODUCTION

IN the first paper of this series (Wardlaw and Leonard, 1936) it was suggested that, in studying the phenomenon of respiration in fruits, attention should be given not only to the measurement of carbon dioxide liberation and oxygen intake at the surface of the fruit but also to the concentrations of these gases present in the intercellular air spaces or central cavity where present. In support of this view it was shown that during the development, ripening, and senescence of several tropical fruits definite trends in the internal concentrations of carbon dioxide and oxygen could be demonstrated by simple means. In particular, in the papaw—a fleshy fruit with a large central cavity—it was found that the concentration of oxygen is relatively low during the early stages of development, that it reaches a peak value just prior to the onset of ripening, and that subsequently during senescence it diminishes to very low values. It was also shown that during those stages of development and ripening when the internal concentration of oxygen is relatively high, the value for the internal concentrations of carbon dioxide plus that of oxygen is approximately 21 per cent., as in the composition of normal air, but that where the concentration of oxygen falls to a low value, as during senescence, the value of carbon dioxide plus that of oxygen is less than 21 per cent., a value of approximately 16 per cent. commonly coinciding with these low oxygen values. These observations direct attention to the resistance to the movement

of gases which fleshy tissues may offer, particularly during certain stages of development and ripening.

At an earlier stage in these investigations it had not been ascertained that differences in pneumatic pressure between the cavity or internal air spaces and the atmosphere existed in young and senescent fruits. By attaching manometers to fruits, however, it has since been demonstrated that negative pressures are found in papaws at certain stages, an observation which suggested that manometric records might prove useful in the study of gaseous interchange in fruits.

In this preliminary account data are submitted which indicate a relationship between such manometric records and the internal concentrations of carbon dioxide and oxygen during the development, ripening, and senescence of papaw fruits. Observations on other tropical fruits including the banana and mango have also been made. These show certain divergences from the trends observed in the papaw, and will be the subject of later communications.

II. EXPERIMENTAL METHODS

In the experiments described below papaws at different stages of development and ripeness were used. The methods adopted in estimating the internal concentrations of carbon dioxide and oxygen in small and large papaws and other fruits have already been described in detail (Wardlaw and Leonard, 1936, p. 625). In the determination of internal gas concentrations the convenience of using large fruits with considerable cavities is apparent.

It is realized that while the gaseous concentrations observed in the cavity are a close approximation to those in the immediately adjacent intercellular spaces, slightly different concentrations may occur in those of the more distant tissues. On the other hand, where the removal of gas samples from relatively compact tissues also involves the withdrawal of gases from solution in the tissues due to the reduction in pressure, the estimations obtained may not give a correct value for the internal concentrations; it is typical of such methods that the values for carbon dioxide plus oxygen recorded are generally in excess of 20.96 per cent. (Magness, 1920; Dowd, 1933.) Nevertheless such high values do exist in certain fruits. They will be the subject of further papers in this series.

In order to observe the pneumatic pressures within the cavity of a papaw and to be able to withdraw samples of gas for analysis at intervals, fruits were fitted with gas-sampling tubes attached to a three-way stopcock of capillary tubing (Fig. 1). One arm of the stopcock was joined to a narrow-bore manometer tube containing medicinal paraffin, which gives a sufficiently sensitive manometer without introducing the risk of evaporation or of effects harmful to the fruit.¹ The other arm was connected direct to the Haldane gas

¹ Specific gravity of medicinal paraffin at 30° C. = 0.8810; 1 cm. is equivalent to 0.8847 cm. water, or 0.0652 cm. Hg at 30° C.

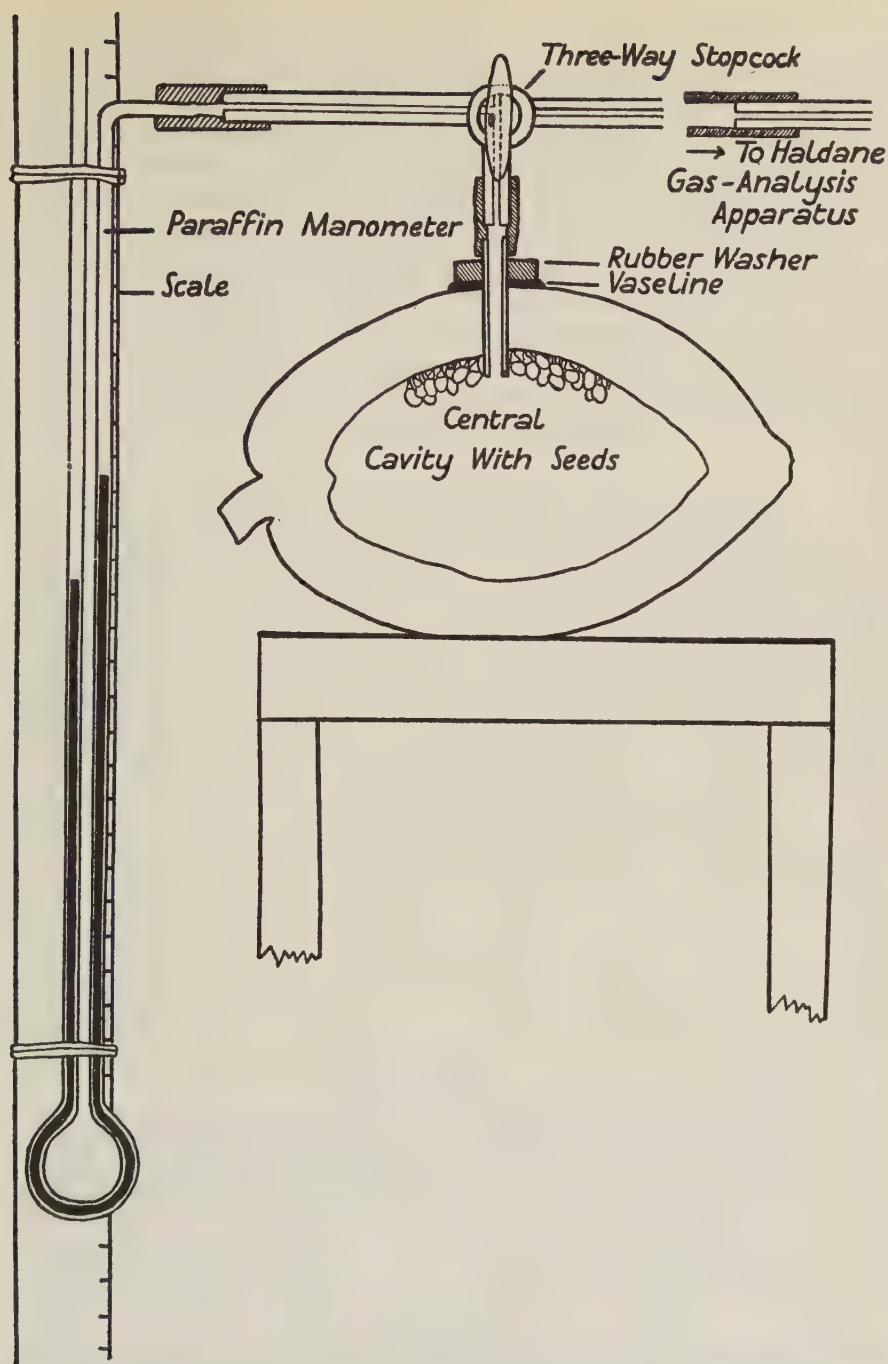


FIG. 1. Arrangement of apparatus for determining internal concentrations of carbon dioxide and of oxygen and also of pneumatic pressure in papaw fruits. The sampling tube, inserted into the fruit cavity, is attached by a three-way stopcock to a paraffin manometer or to the Haldane gas apparatus, as required.

apparatus whenever samples of the fruit cavity gases were being withdrawn for analysis, the manometer meanwhile being shut off.

For observations of internal pressure and gas concentrations during development, a series of fruits of increasing size, from small fruits just pollinated to full-grown green fruits, was gathered at the same time from a single tree. Observations during ripening and senescence were based on single, full-grown fruits, harvested when showing the presence of some ripening colour. Initially the manometer was adjusted to equilibrium with the atmosphere by opening the stopcock, but after each withdrawal of a gas sample, in the case of ripening fruits, it was put back into connexion with the cavity without readjustment.¹ Vertical lines on the graphs represent the points at which samples were taken; the manometer readings before and after are given.

III. INTERNAL PRESSURES DURING DEVELOPMENT

Young developing papaw fruits are characterized by relatively high internal concentrations of carbon dioxide, with concomitant high rates of liberation of carbon dioxide from the fruit surface, and by low oxygen concentrations. During development to the fully-grown pre-climacteric stage, the internal carbon-dioxide concentration diminishes and the oxygen concentration increases. Fig. 2 shows generalized curves of the internal concentrations during such a developmental sequence for carbon dioxide, oxygen, and carbon dioxide plus oxygen. A generalized curve for the corresponding internal pressure as measured by paraffin manometers is also shown. Figs. 3 and 4 show in detail the data from which the curves in Fig. 2 have been derived.

It will be seen that in small immature fruits a considerable negative pressure is present and that, in general, there is a very close similarity between the manometer curve and those for the internal concentrations of oxygen and of carbon dioxide plus oxygen. It has already been argued (Wardlaw and Leonard, 1936) from the occurrence, in young fruits, of a steady high internal concentration accompanied by a high rate of liberation of carbon dioxide, that the compact young carpellary tissue offers a high resistance to the movement of gases.

As the fruits increase in size, the constituent cells enlarge as do also the intercellular spaces. This process is accompanied by a diminution in the internal concentration of carbon dioxide and of the rate of liberation of this gas, with a colligative increase in the internal concentration of oxygen. Preliminary experiments on plugs of tissue and on whole fruits showed that this developmental sequence is accompanied by decreasing resistance to gas movement, i.e. indicating increasing porosity (Wardlaw and Leonard, 1936).

¹ It may be noted that the maximum diurnal variation of the barometric pressure, as recorded by microbarograph in Trinidad, is 5 mm. Hg., the normal value being 762 mm. Since, however, in the system adopted, the manometer connected to the cavity of fruits exposed on the bench had also an open arm exposed to the air, small changes in barometric pressure were compensated for.

The results of the manometric observations confirm these earlier results and further elucidate the changes in tissue resistance.

It will also be noted that the point of transition from negative to positive pressures coincides closely with that point at which the sum of the values of

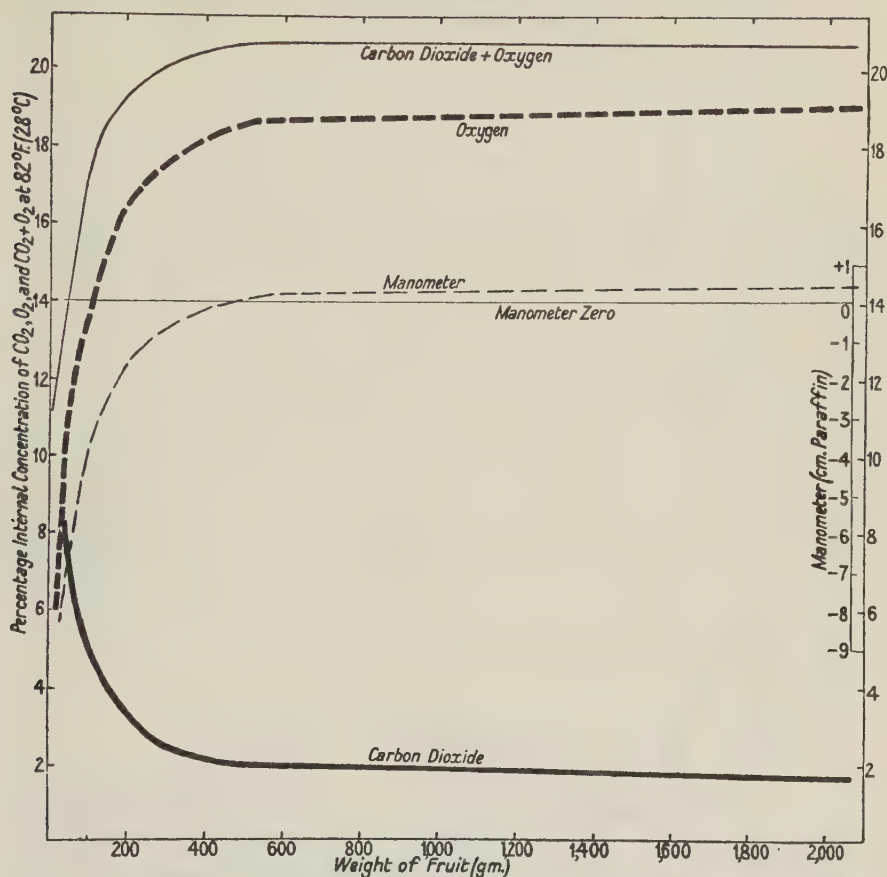


FIG. 2. Generalized representation, based on the data shown in Figs. 3 and 4, of the relationship between the internal concentrations of carbon dioxide, of oxygen, and of carbon dioxide plus oxygen, and of the pneumatic pressure as recorded by paraffin manometers, during the development of papaw fruits.

the internal concentrations of carbon dioxide and oxygen approximates to 21 per cent.

Figs. 3 and 4 may now be considered in greater detail. It will be noted, in the two lots of fruit (each taken from a single tree and held at the same constant temperature) that smooth curves have not been obtained, although the individual observations clearly indicate the general trend. These departures from the smooth curve are not due to experimental errors in the determination of the gas concentrations, the extent of errors in using the

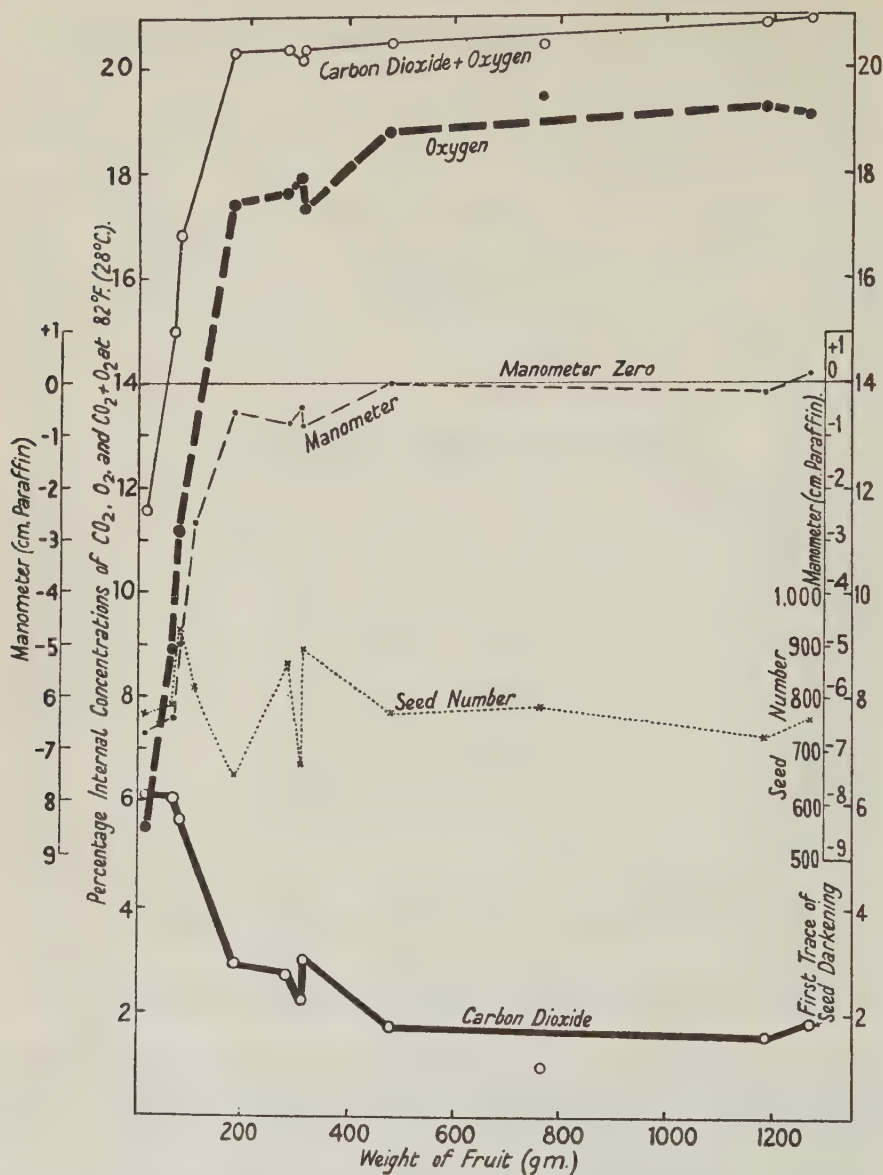


FIG. 3. Internal concentrations of carbon dioxide, of oxygen, and of carbon dioxide plus oxygen, of pneumatic pressure and number of seeds, in a series of developing papaw fruits (Porto Rico variety) harvested at the same time and held at a constant temperature of 28° C. (The ninth fruit in the series, weighing 766 gm. yielded the aberrant points shown in the graph.) The largest fruit shown in this series was a little more than half-grown.

Haldane apparatus being very small indeed; they are, in fact, individual variations which will be discussed later. It will be noted that the fluctuations in the oxygen curves are accompanied by corresponding fluctuations in the

manometer and carbon-dioxide curves, those in the carbon-dioxide curve being in the opposite direction to those of the oxygen curve.

It has already been postulated (Wardlaw and Leonard, 1936) that during the growth of the papaw fruit to adult size the development of the numerous

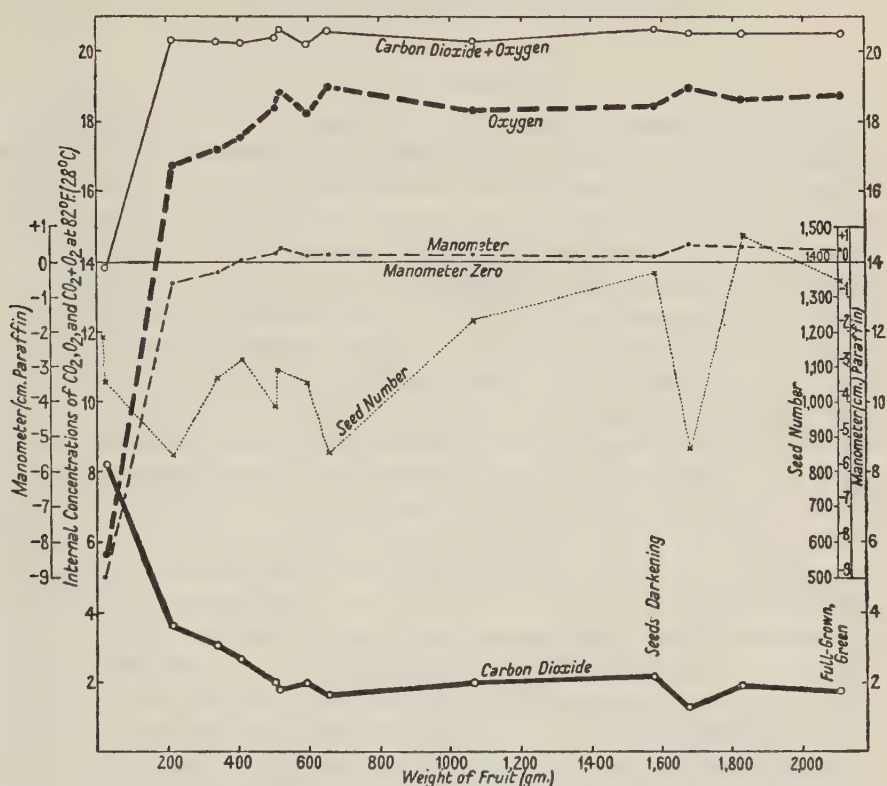


FIG. 4. Observations, as in Fig. 3, on papaws of the Porto Rico variety. The largest fruit in this size series was full-grown, i.e. at the pre-climacteric stage of maturity.

seeds abutting on the large central cavity exercises a marked effect on the internal gas concentrations, the peak oxygen value coinciding with the final maturation and induration of the seeds. The hypothesis that the irregularities observed in the several curves might be due to variations in the numbers of seeds¹ present in the fruits was tested by plotting these numbers alongside the other curves, with the results shown in Figs 3 and 4. With possible exceptions among the very youngest fruits, the indentations in the seed-number curve correspond closely with the indentations in the curve of internal carbon-dioxide concentrations and are inversely related to the curve of internal

¹ During development the stalk of the papaw seed undergoes a remarkable enlargement at the distal end; changes associated with seed development, and in particular respiratory variations, are therefore accentuated.

oxygen concentration, i.e. high seed numbers correspond with high internal carbon dioxide and low oxygen concentration.

As fruits develop to full size it will be seen that the curve of carbon dioxide plus oxygen concentrations approximates closely to 21 per cent. and that the manometer curves ascend from considerable negative values to small positive values of approximately 1–2 cm. of paraffin.

IV. INTERNAL PRESSURES DURING RIPENING AND SENESCENCE

Considerable information has been collected on the internal gas concentration and pressures occurring in fully grown papaws picked at different stages of maturity. In each instance the graphs submitted represent successive readings from single fruits. Observations were made as detailed above (section II). In addition flesh temperatures were observed by mercury thermometers (reading to 0.2° F.) inserted into the flesh so that the bulb was inside the cavity and in contact with the opposite side of the cavity. Figs. 5, 6, and 7 show internal concentrations of carbon dioxide, oxygen, and carbon dioxide plus oxygen, flesh temperatures, and manometer readings for three papaws, A₁, A₂, and A₃ harvested green, greenish-yellow, and yellow respectively, all being taken from the same tree at the same time. During the first two days the fruits were held at laboratory air temperatures which fluctuated between 75° and 95° F. (24°–35° C.), and afterwards at a uniform temperature of 75° F. (24° C.).

In the green fruit, Fig. 5, the internal concentrations of carbon dioxide and oxygen were relatively slightly affected by a rise in flesh temperature, and the value of carbon dioxide plus oxygen concentrations remained approximately 21 per cent. Since respiration rate increases with rise in temperature, the indication is that gases can pass through the tissues of fruit of this maturity with considerable ease. Support for this view has been obtained from other experiments not described. In the colouring and yellow fruits, Figs. 6 and 7 on the other hand, a rise in temperature caused marked changes in the internal gas concentrations, carbon dioxide increasing and oxygen decreasing, and the value for the sum of the concentration of carbon dioxide and oxygen falling below 21 per cent. in the more mature fruit (A₃). It will be observed that the trend of the manometer curve in each case follows closely that of the oxygen and of the carbon dioxide plus oxygen curve; these results for ripening fruits are therefore in conformity with those recorded above for developing fruits.

In these studies attention has been directed to the fact that as a papaw ripens and its tissues become soft, increased resistance is offered to the movement of carbon dioxide outwards and oxygen inwards, the final result in the later stages of senescence being the more or less complete exhaustion of the cavity oxygen. It has been suggested (Wardlaw and Leonard, 1936) that oxygen, though still passing inwards, under a progressively increasing gradient, no longer enters at a sufficient rate to keep pace with metabolic requirements. Carbon dioxide, due to increased tissue resistance, rises to higher internal

concentrations in spite of high rates of liberation, but gaseous movements are so impeded that diminished pressures occur internally. This is discussed more fully later. The manometric observations recorded in Figs. 5, 6, and 7 give further support to views on tissue resistance which have been advanced.

Some further points may now be considered in detail.

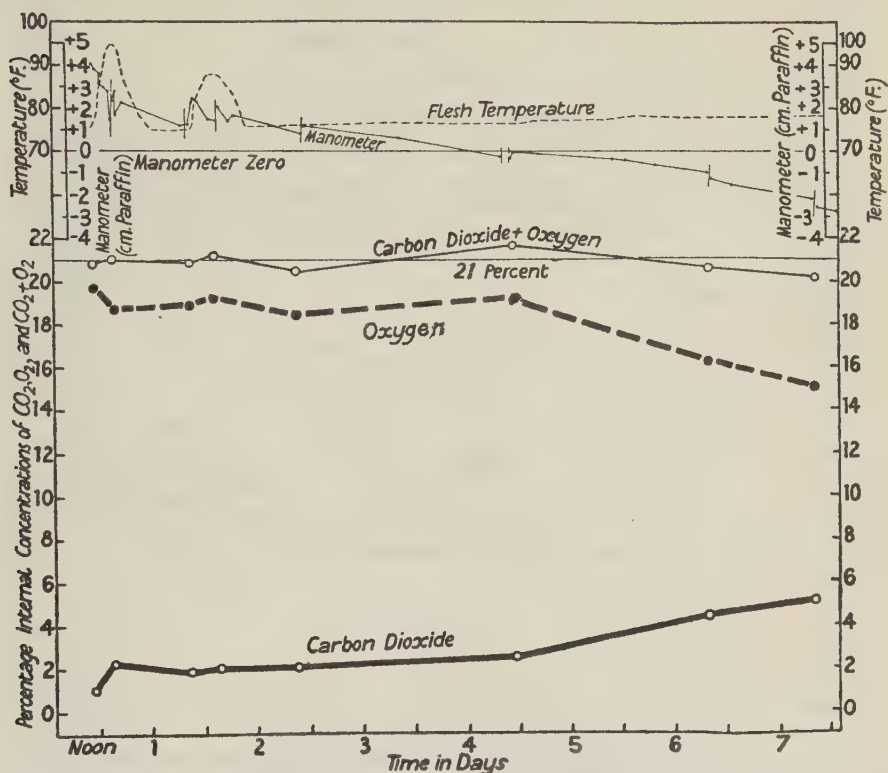


FIG. 5. Internal concentrations of carbon dioxide, of oxygen, and of carbon dioxide plus oxygen, pneumatic pressure and flesh temperature in a papaw fruit (A1) harvested green but full-grown and held at a uniform temperature of 75° F. after two days at a temperature fluctuating between 75° and 95° F. Vertical lines on the manometer curve indicate points at which samples of cavity gas were withdrawn. Data for the final stages of ripening are not included in this graph.

Green fruit

In the pre-climacteric, green fruit, Fig. 5, a positive pressure, i.e. one higher than the surrounding atmosphere, was recorded within the central cavity until that stage in maturation was reached at which departure from 21 per cent. carbon dioxide plus oxygen occurred, following diminution in the percentage of cavity oxygen. It should be noted that this positive pressure was regained almost instantaneously after each withdrawal of a 10 c.c. sample of the cavity gas; in fact, a *higher* pressure was temporarily recorded as a result of an operation which, in a correspondingly purely physical system,

composed of a closed, non-porous vessel, would produce a *diminished* pressure. As a partial explanation of this phenomenon it may be suggested that since the flesh temperature, due to respiratory activity is higher than that of the

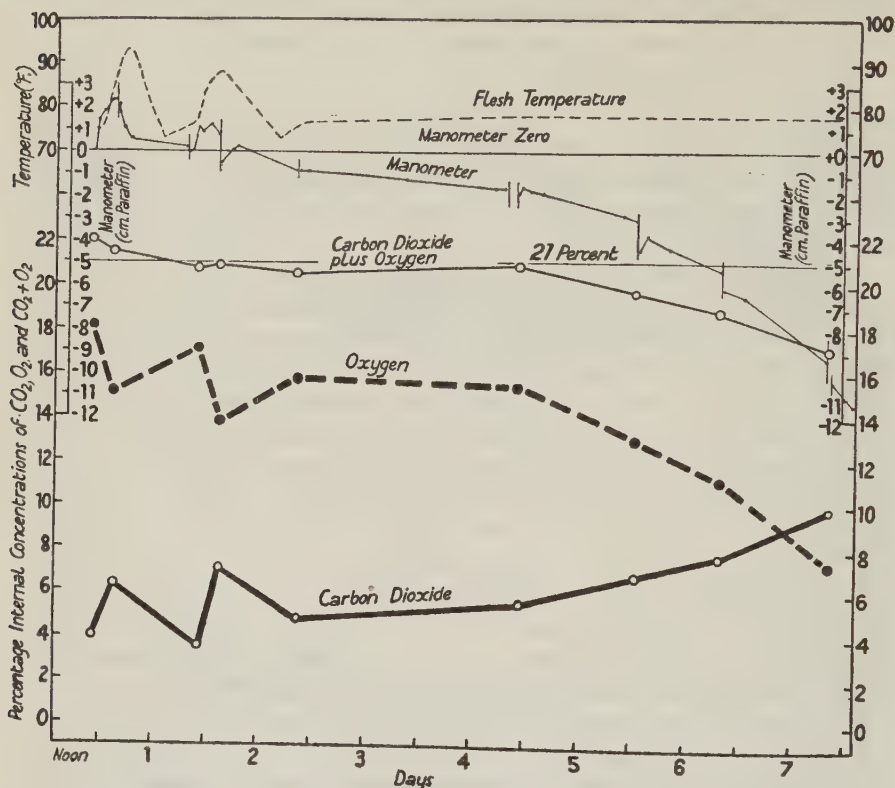


FIG. 6. Observations as in Fig. 5 for a papaw fruit (A2), harvested greenish-yellow, and subject to the same storage conditions.

surrounding air, any gas drawn into the cavity by a temporary reduction of pressure will expand and produce a positive pressure inside the fruit.¹

Zero pressure difference (i.e. equilibrium with the atmosphere) within the fruit approximately coincides with the point in maturation at which the sum of the internal concentrations of carbon dioxide and oxygen falls below 21 per cent. From this point onwards there is a steady decrease in the internal pressure and withdrawal of a sample produces an increase in negative pressure.

¹ A consideration of the effect of the difference between air and flesh temperatures on pressure, expressed in terms of cm. paraffin, as in the manometric observations recorded, supports this view. To take a simple example, a positive manometer difference in pressure of 2 cm. paraffin; approximately equivalent to 1 mm. Hg, would be produced by a difference between flesh and air temperature of 0.4° C. at 25° C.; such differences have been observed.

Colouring and full yellow fruit.

The change from zero to negative pressure is shown more clearly in Fig. 6 for papaw A2 picked greenish-yellow; its occurrence at about the point of departure from the 21 per cent. value for carbon dioxide plus oxygen will

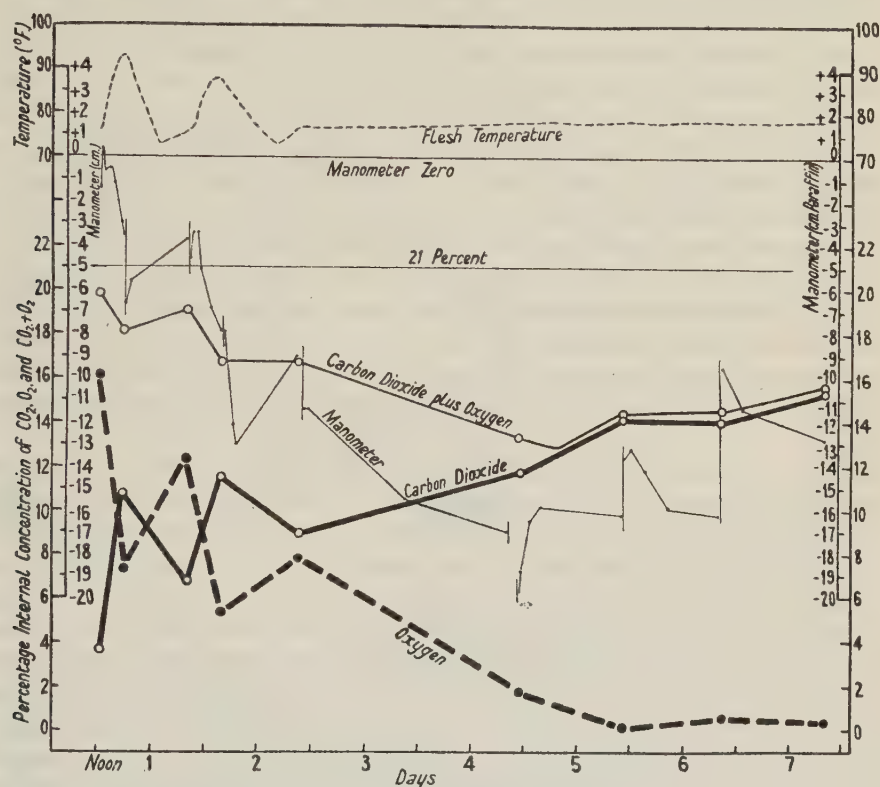


FIG. 7. Observations as in Figs. 5 and 6 for a papaw fruit (A3) harvested when yellow and almost ripe, and subject to the same storage conditions.

again be noted. In the yellow fruit, A3 (Fig. 7), this sum value was less than 21 per cent. at the commencement of observations and the manometer registered negative pressures throughout the experiment apart from a very slight rise with the initial temperature increase on the first day. The result of withdrawing a sample of gas from the cavity of a papaw whose internal carbon dioxide plus oxygen concentration has fallen below 21 per cent. is thus in accordance with the purely physical system previously envisaged, up to the point, in late senescence, at which the internal oxygen concentration falls to very low values, and the carbon dioxide plus oxygen value commences to rise. This decreased internal pressure on withdrawing a sample of gas is taken to be an indication that the permeability to gases of the tissues of a ripening papaw is approximating to that of the non-porous vessel and

that this is the controlling factor in the regulation of the internal gas concentration and pressure.

There remains the problem of the trend of decreasing internal pressure with increasing senescence. It will be observed that in Figs. 6 and 7 the downward trend of the internal oxygen concentration is closely paralleled by the manometer readings. It is suggested, therefore, that it is the utilization of cavity oxygen in metabolism, coupled with the decreased rate of replacement of this gas due to increased tissue resistance, which causes the observed increasing negative pressures. Partial compensation of the reduced pressures developed will occur as a result of (a) the effect of the flesh temperature as detailed above for the green fruit, and (b) gases, including water vapour, coming out of solution, so that the negative manometer reading is a resultant of these processes, the increased tissue resistance being apparently the major factor. The fact that the negative pressure is not completely compensated by (b) may be taken as an indication that both water and gases are retained in the tissues under active protoplasmic control up to a certain stage in senescence. In this connexion reference may be made to recent views on the special properties of protoplasm in controlling physical function (Phillis and Mason, 1937).

In Fig. 7 fluctuations in flesh temperature during the first two days are seen to be accompanied by considerable alterations in internal carbon dioxide and oxygen concentrations, carbon-dioxide percentage rising and oxygen percentage falling with increase in temperature and vice versa. During the subsequent period at a steady air temperature of 75° F. the internal oxygen concentration falls asymptotically towards zero, while the manometer follows a parallel course to about the point at which the internal carbon dioxide concentration reaches 13 per cent. and oxygen less than 1 per cent. From this point onwards increasing manometer readings, i.e. decreasing negative values, are found and the carbon dioxide plus oxygen curve rises; also withdrawal of a sample of gas is followed by a rise in manometer reading, i.e. a decrease in negative pressure. Without entering on a detailed discussion it is suggested that the upward trend of the manometer curve during the final phase of senescence (Fig. 7), accompanied by a rising concentration of carbon dioxide, represents a progressive decline in the protoplasmic control of retained gasses.

To eliminate the possibility of disturbing effects on the normal metabolism of the removal of samples of gas from the cavity during ripening two comparable¹ papaws from the same tree, showing the first traces of ripening colour, were selected for study and kept at a uniform temperature of 78° F. The internal cavity of one was attached direct to a manometer and the fruit left untouched until the end of the experiment; the other was used for the measurement of internal gas concentrations. The full experimental record is shown

¹ Not only did the two fruits match closely in the matter of weight and development of colour during ripening, but both developed anthracnose spots on the same day.

in Fig. 8. As with the papaws in the previous experiment (A₁, A₂, and A₃), at the point in time when the sum value for the internal concentrations of carbon dioxide and oxygen fell below 21 per cent. in the one fruit, the manometer attached to the other indicated that a progressive development of a

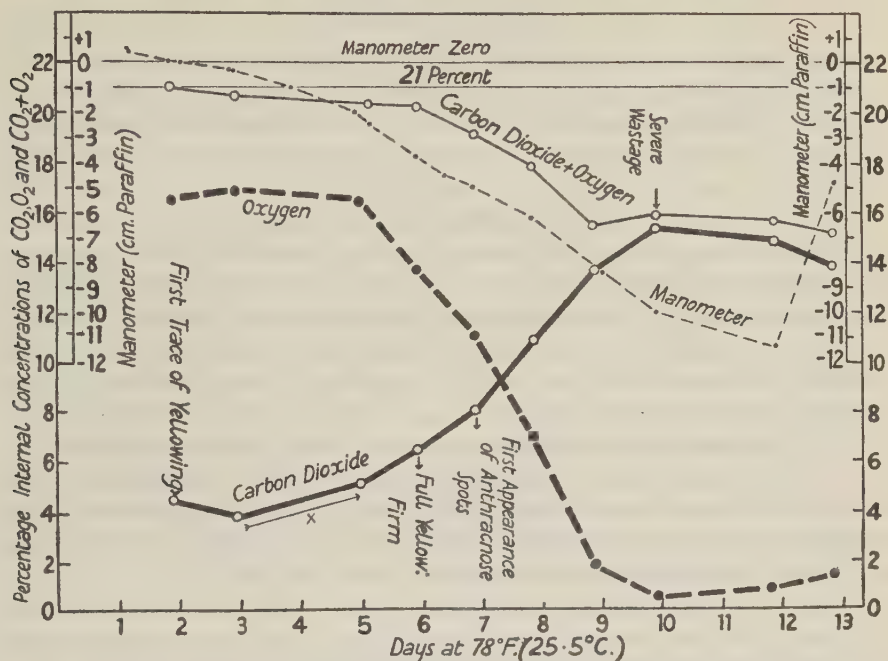


FIG. 8. Observations on two strictly comparable papaw fruits, harvested when showing the first trace of yellow colour in the skin, and ripened at a constant temperature of 25.5° C. One fruit was attached to a paraffin manometer and left undisturbed until the end of the experiment, the other was used for the determination of the internal concentrations of carbon dioxide and oxygen during ripening. Important changes in the external appearance of the skin are noted; the arrow at X indicates approximately the period during which the climacteric rise in carbon-dioxide liberation at the fruit surface takes place.

negative pressure was taking place within the fruit cavity. The curve of internal carbon-dioxide concentration is annotated to show the colour change and wastage incidence. It will be observed that the first appearance of anthracnose spots (*Colletotrichum gloeosporioides*) occurred when the internal concentration of carbon dioxide was 8 per cent. and the oxygen concentration was 11 per cent., i.e. a carbon dioxide plus oxygen value of 19 per cent. Severe wastage due to secondary fungi as recorded in earlier experiments (Wardlaw and Leonard, 1936), occurred at the stage at which the internal oxygen concentration had almost reached zero value; at this stage the carbon dioxide plus oxygen value was 16 per cent. The manometer showed continued increasing negative values until a late stage in senescence.

It has already been shown (Wardlaw and Leonard, 1936) that, in the later stages of senescence, some variability may be encountered in the trend of the

internal gas concentrations; it is suggested that the different rates of senescence and decline in individual fruits and the nature and rate of exploitation of the tissues by secondary fungi account for the variability observed.

V. DISCUSSION

In the experiments described above confirmation has been obtained of the hypothesis previously put forward (Wardlaw and Leonard, 1936) that the resistance which the tissues of fruits offer to the movement of gases during development and senescence is of considerable importance in the study of respiration.

The experiments described in the application of manometry in fruit ripening studies are regarded as being of a preliminary nature only, and no attempt will be made at this point fully to account for the observations recorded. Manometric pressure is the resultant of many interacting factors, including (a) the rates at which the several gases pass into and out of the fruit, (b) their solubility and rates of solution and de-solution, (c) their partial pressures within and outside the fruit, (d) the rates of oxygen utilization and carbon-dioxide liberation, (e) the difference between flesh and air temperatures, (f) the level of protoplasmic organization, and (g) the tissue content of carbon dioxide. In so far as the manometer affords an additional means of investigating this complex of factors, which is also involved in respiration, it is suggested that its use may be extended as indicated in these studies.

It may be emphasized that the general trends of internal concentration of gases and of internal pressure demonstrated have been consistently obtained with the papaw. It may be anticipated that different types of metabolism, as exemplified by different kinds of fruit, will show distinctive differences, and, in fact, such differences have already been found with other tropical fruits, e.g. the banana and mango, upon which preliminary observations have been made.

While measurements of pneumatic pressures in the tissues of plants have been made by various workers dealing with other aspects of plant physiology, the writers believe that the preliminary observations recorded here represent the first direct application of manometric methods to respiration in fruits during development and ripening. In this connexion it is relevant to record an observation by Gustafson (1930) in which he puts forward the suggestion that a possible consequence of irrigating tomato fruit with a rapidly diffusing gas, such as hydrogen, would be to create an increased internal pressure.

VI. SUMMARY

1. Further observations with reference to respiration have been made on the changes in the internal concentrations of carbon dioxide and oxygen during the development, ripening, and senescence of the papaw.
2. By attaching sensitive manometers to several different tropical fruits at different stages of development and ripening it has been shown that con-

siderable departures from the normal atmospheric pressure are readily demonstrated.

3. The manometer records obtained show definite trends which may be used in the elucidation of respiration phenomena in fruits. By fitting manometers to the cavities of papaws during development and ripening it has been shown (i) that immature, developing fruits at normal air temperatures are characterized by a negative pressure; (ii) that full-grown green fruits are characterized by a small positive pressure; (iii) that as ripening proceeds, there is a progressive decrease in this positive pressure until, in senescent yellow fruits, a definite negative pressure is produced; and (iv) in final senescence there is a recovery towards atmospheric pressure.

4. It has been observed in the papaw fruit that the transitions from negative to positive pressure, and vice versa, coincide almost exactly with those stages in development and ripening respectively at which the sum of internal carbon dioxide and oxygen concentrations rises to or falls from (approximately) 21 per cent.

5. The results obtained lend support to views previously expressed that during development, ripening, and senescence, the tissues of fleshy fruits offer a changing resistance to the movement of gases.

6. Reasons have been advanced in support of the view that the manometer record, being the resultant of the several gaseous phenomena involved in metabolism, is of considerable value in the study of respiration.

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Effects of Age and of Season upon Protein Synthesis in Detached Leaves

BY

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THE problems of protein synthesis form a fundamental part of plant metabolism in the sense that they deal with substances which appear to be necessary for the existence of protoplasm. It is thus natural that these problems have given rise to a large amount of research. It is equally true that most of the problems involved in protein synthesis in plants are yet unsolved, and indeed, the results obtained are hardly commensurate with the immense amount of work that has been contributed to the subject. A wide review of the literature suggests that investigators have followed in essence the methods found to be adequate in the study of carbohydrate metabolism in leaves, and that many, at any rate, have expected to find protein accumulating in illuminated leaves in quantities similar to those of the higher carbohydrates. The results have shown, however, that the daily drifts in protein content are small in attached leaves (see particularly Chibnall, 1924, and Miller, 1931), so that if rapid protein synthesis does take place it must be paralleled by a rate of translocation of the same order of magnitude. The study of protein formation in attached leaves is, then, largely a question of obtaining significant differences, and it is almost too much to hope that the measurements can be made sufficiently refined to throw much light on the general metabolic processes involved. Any technique used for studying these processes must, therefore, aim firstly at obtaining a high rate of protein formation.

In this connexion the advantages of using detached leaves are obvious, even without such previous work as that of Mothes (1926), and a second feature which hardly seems to have received adequate recognition is the importance of the previous history and physiological state of the tissues used. This paper, therefore, outlines some of the problems met with in considering the influence of the latter factors.

MATERIAL AND METHODS

The essential feature of the method employed was to float detached leaves of different ages upon nutrient solutions which allowed of rapid protein synthesis. Representative samples of the different leaves used were kept for

analysis so as to define their original condition. The experimental leaves were analysed at the same time and under similar conditions so that each series is exactly comparative.

To obtain leaves of different ages the method used by Pearsall (1931) and by Hover and Gustafson (1926) was employed, in which the successive leaves on shoots (from the apex downwards) were taken to represent an age series. This method is not applicable to linear-leaved Monocotyledons, and in the experiments on daffodils (*Narcissus*) and *Iris*, individual leaves of similar size and appearance were divided into a series of segments of similar length, and grouped in order from the apex downwards. In these leaves the base remains meristematic (and colourless) and the apex is the oldest part of the leaf. An ordered series of segments thus represents an age series. The leaves used were in each case taken from a large batch of similar plants growing in one plot, so that the possible effects of varying soil and light factors were uniform. The method of representing age has the advantage that the different age units are taken under parallel conditions of light, temperature, and food-supply, only the age varying. It is obvious, however, that the older leaves in any such series may not have developed under conditions similar to those existing at the time of sampling and there may be a physiological drift as the conditions of growth alter with the age of the plant and the season (cf. Culpepper and Caldwell, 1932, and Richards, 1934). Examples of this drift will be considered.

The method of sampling was to take a minimum of twenty leaves from corresponding nodes on similar shoots. Larger numbers were often necessary in the case of young leaves. The average leaf-length per node on the shoots was determined previously on other plants and the leaves taken had lengths within 1 cm. of the average for the particular node. Any leaves of abnormal size or appearance were discarded. Daffodil and *Iris* leaves of similar age, length, and appearance were selected.

Normally half of each leaf was immediately prepared for analysis, the remainder being floated on the nutrient solution. Monocotyledon leaf segments were split longitudinally. In a few cases (plants with opposite leaves) the twin leaf method was employed, in which symmetrical and similar sized opposite leaves are assumed to be equivalent (see Denny, 1932) and treated as halves of one leaf. The precautions recommended by Thoday (1909) and by Denny (1932) were observed.

The standard nutrient solution used was one containing 3 per cent. of glucose and 0.2 per cent. ammonium nitrate. Preliminary observations had established the fact that this mixture was the one giving the largest amounts of protein synthesis. The data for these are not given in full, though some typical results (Tables II, III, IV) indicate the type of experiment used. The nutrient medium was maintained at pH 6, by adjustment every twelve hours with 0.1 NaOH, bromthymol blue being used as indicator. To reduce the chance of bacterial or fungal infection, the leaves were first washed with

freshly prepared calcium hypochlorite (6.66 per cent.) and then with sterilized distilled water (see Wilson, 1915). The medium was changed daily, and the experiments set up in large covered vessels like deep Petri dishes. In some later experiments, a phosphate buffer solution at pH 6 was used instead of the frequent adjustments of pH. No significant difference in effect was observed between buffered and unbuffered media. To facilitate temperature control, the experiments were usually carried out in the dark. The effects of light will be considered separately. The temperature at which the experiments were conducted was 15–17° C.

As the subject to be investigated involved the measurement of gains or losses of protein, it was obvious that the methods used must at least be capable of allowing significant differences in protein content to be measured. Various methods of treatment were possible in separating the protein fraction, but those involving washing out the soluble constituents with water were open to the disadvantage that some of the protein and proteose nitrogen is also extracted. This has to be removed from the extract separately and, for convenience, it is usually separately estimated. The presence of this fraction is, however, a disadvantage in experimental work as its variation seems to be unimportant, and complication of procedure is undesirable if the methods are to be capable of dealing with comparatively small samples of tissue. For this reason the method of alcohol extraction seemed preferable, the principle involved being that of precipitating the proteins in the tissue (see Osborne, Wakeman, and Leavenworth, 1921). Moreover, alcohol also extracts chlorophyll nitrogen, the principal type of non-protein nitrogen insoluble in water. The use of 70 per cent. alcohol then leaves an insoluble nitrogen fraction which is possibly the closest approximation to the true protein nitrogen. The methods of extraction used were:

Method A. A standard method for the extraction of soluble carbohydrates employs hot alcohol. In some series of observations the extraction of soluble nitrogen was carried out by plunging the finely cut-up leaves into boiling 70 per cent. aldehyde-free alcohol and boiling under a reflux for one hour. The extract was then separated, the residue dried, finely ground and stored in a desiccator. The nitrogen content of each fraction was estimated separately.

Method B. A convenient method in experimental work with small quantities of tissue is the drying method. In this the leaf sample, well spread out with the lower surface uppermost and freed from external moisture, is heated in a well-aired oven to 95° C. for half an hour and then dried at 65° C. to constant weight. This method has been found suitable in certain cases for carbohydrates by Link and Tottingham (1923) and for nitrogen fractions by Pearsall and Wright (1929). Much depends on the speed of the initial heating and the aeration. Properly dried leaves should be a clear green, and this is a good rough test of the suitability of the method for a particular tissue. The dry material is then finely ground and stored in a desiccator. It is extracted by standing in cold 70 per cent. alcohol for twenty-four hours or

until the whole of the pigment is extracted. The alcohol is used in the proportion of 1 c.c. to 4 mg. of tissue, and is replaced after twelve hours. The nitrogen contents of the extract and of the unextracted powder are estimated, protein N being obtained by difference.

Method C. Fresh leaf material is finely minced in the presence of a mixture of equal parts of ether and xylol and then immediately placed in cold 70 per cent. alcohol (containing 10 per cent. of ether), in the proportion of 15 gm. to 100 c.c. of solvent. The alcohol is changed after twelve hours and the extraction continued for twenty-four hours or until the tissue is colourless. The residue is dried, powdered and stored in a desiccator. The nitrogen contents of extract and of residue are determined. The method has been used with daffodil, Coleus, Iris, Lupinus, and Pelargonium leaves.

Method D. The leaves are etherized, finely minced, and the pulp extracted and pressed after washings of water saturated with ether. At least six washings are normally desirable. These are carried out as rapidly as possible. The residue is dried, powdered, and stored for protein nitrogen determinations. This method was only used for privet (*Ligustrum*) leaves, which showed marked pigment discoloration on drying or on extraction with hot alcohol.

On account of the fact that the alcohol methods extract pigments as well as other soluble materials, they cannot easily be compared with the aqueous extraction method (D). The cold alcohol method was devised first as a substitute for method D, but the presence of pigments in the first extracts showed that the logical end point of the extract ought to be complete removal of pigment. By comparison of this method as described above with water extraction (method D) followed by acetone or alcohol extraction of pigments, it appears that the cold alcohol extracts less nitrogen than does water followed by pigment extraction (cf. Billimoria, 1936). In other words, some component of the soluble nitrogen fraction is not extracted by cold alcohol. This component is possibly the proteose and water-soluble protein fraction. The fact shows at the same time that no pronounced hydrolysis of protein takes place on standing in cold alcohol. The extra nitrogen extracted on standing appears to be entirely pigment nitrogen. Comparison of replicate extractions by the methods A, B, and C on the same batch of leaves (*Daffodil* and *Tropaeolum*) shows an agreement in protein nitrogen to within 4 per cent. This is so nearly the sampling error (see p. 322) that it is probably unjustifiable to draw conclusions as to the relative efficiencies of the methods on suitable materials. The drying method though convenient should certainly be used with caution, and particular attention should be paid to signs of discoloration after drying. This method is quite unsuitable for acid tissues (*Pelargonium*, *Ligustrum*) owing to discoloration and hydrolysis, but it is doubtful whether any method entirely prevents hydrolysis in such tissues. Hydrolysis can be shown to exist when hot alcohol is used and the smallest yields of soluble nitrogen are given by cold alcohol with ether present. We attach considerable value to the colour

of the leaf pigments after extraction as a guide to possible post-mortem changes. On this basis, the cold alcohol method as used appears to be the best of those tried. We assume that enzymes are inactivated by the solvents employed.

Estimation. Nitrogen estimations were carried out by the Kjeldahl method, or if nitrates were present by Ranker's modification (1928). The estimations were done in triplicate in the Pregl micro-apparatus using quantities of nitrogen of the order of 0.5 mg. With good technique a high order of accuracy is possible with this apparatus and replicates should normally agree to within at least 0.01 mg. if not to within half this quantity. Exact standardization of the quantities of reagents and of procedure is necessary. Materials for analysis should be thoroughly dried on a water bath and fine powdering of solid residues is necessary to get homogeneous material for analysis.

EXPRESSION AND SIGNIFICANCE OF RESULTS

The method of expressing the results must necessarily take into account the fact that the leaves may gain in dry weight, in water content, and in total nitrogen content during the experiment. The results of changes in composition can thus be expressed only on the area or on the original fresh weight, and the latter proved to be by far the simplest in dealing with large numbers of leaves. Area was used in some of the earlier experiments. This method is suitable if the leaves are uniform in thickness, but in daffodil leaves, for example, some variation in thickness may occur between the apex and base, so that the results for different segments may be less exactly comparable. The method of estimating area with a planimeter gave variable results with the small leaf segments used and areas were therefore measured by tracing the leaf segment on squared paper, a laborious method when large numbers of segments are used.

The differences in insoluble N observed between similar samples of leaves using the normal technique are given in the following table, in which the difference between the replicates A and B is given as a percentage of B. The data for primary leaves (i.e. first green leaves) of *Phaseolus vulgaris* are given, as the nitrogen content of these leaves may change rather rapidly (unpublished results), and hence sampling errors may be large unless the technique is good.

It will be seen from the results given in Table I that the percentage difference between the estimates does not exceed 3. It is assumed, therefore, that differences in protein (insoluble) nitrogen are significant if they exceed this value. Denny (1932) obtained a similar though slightly higher degree of difference for the half-leaf method and a rather better agreement between replicates by the twin-leaf method.

Results. The first results to be considered will be three series illustrative of the effects of varying the composition of the nutrient medium upon which the leaves are floated. The data are given in the text, as gains or losses of protein nitrogen, as a percentage of that present in the leaves before the

experiment. A difference of more than 3 per cent. should be significant. The actual data are given in the appendices. In each series, the parallel columns are for leaves from the same plants and of similar appearance and age.

TABLE I

Insoluble Nitrogen as Mg per 100 gm. of Fresh Weight. (Half-leaf and Drying Methods)

<i>Narcissus.</i>	Sample A.	Sample B.	Difference as per cent. of A.
Base	127	129	+1.58
	110	107	-2.72
	189	191	+1.06
	346	356	+2.89
Apex	524	521	-0.57
<i>Phaseolus vulgaris</i> (primary leaves)	568	566	-0.35
	580	587	+1.21
	755	776	+2.78
	523	522	-0.19
	485	496	+2.27

Insoluble Nitrogen as Mg. per 100 sq. cm. of Leaf Area (Narcissus, Cold Alcohol and Half-leaf Methods)

Base	9.46	9.26	-2.11
	10.6	10.4	-1.89
	14.7	14.8	+0.68
	21.7	21.8	+0.46
	22.3	22.5	+0.90
Apex	22.6	22.9	+1.33

EFFECT OF VARYING SUGAR CONCENTRATION

TABLE II

Percentage Increase in Protein Nitrogen (Leaf-area Basis)

Segments of Daffodil leaves exposed 72 hours. Methods: half-leaf and cold alcohol.

Segment.	Ammonium nitrate 0.075 per cent.	Glucose concentration (per cent.)	
	1.	3.	5.
1 (Base, i.e. meristematic)	-5.46	9.61	14.1
2	-3.12	1.32	-5.02
3	-12.39	0.51	-5.91
4	-5.60	2.28	-7.85
5	-12.90	-8.92	-13.12
6 (Apex, i.e. oldest)	-5.65	-5.13	-13.98

In this series there is little protein synthesis, except in the youngest segments. Three per cent. glucose tends to prevent proteolysis in the older segments. Five per cent. glucose caused the older leaf segments to become flaccid; this was usually the case. In the large number of leaves examined in this work the internal sugar concentration did not exceed four per cent.

and it was usually much lower, of the order of three per cent. Three per cent. glucose was, therefore, constantly used as the sugar solution.

The limiting factor in protein synthesis in this series was probably the low nitrogen supply. The following series (Table II) was done immediately afterwards on rather longer leaves and is closely comparable with Series I. In this case, the sugar concentration was 3 per cent. and the concentration of ammonium nitrate varied. It will be seen that increasing the nitrogen content extended the number of segments which could manufacture protein. In the maximum amounts of protein manufactured, however, 0.15 per cent. of ammonium nitrate proved somewhat better than 0.2 per cent. As higher concentrations of ammonium nitrate often tended to lead to discoloration or to flaccidity of some of the leaf segments, particularly the younger ones, it was considered that a concentration of 0.2 per cent. was the highest which could be used with safety. Even with this concentration, occasional suggestions of toxic effects were noted.

EFFECT OF VARYING NITROGEN CONCENTRATION

TABLE III

Effect of Varying Ammonium Nitrate Concentration on Percentage Increase in Protein Nitrogen (Leaf-area Basis)

Glucose concentration 3 per cent.; segments of daffodil leaves exposed 72 hours.
Methods, half-leaf and cold alcohol.

Segment.	Concentration, per cent.		
	0.1.	0.15.	0.20
1 (Base, i.e. youngest)	40.17	32.03	31.68
2	12.26	50.22	24.87
3	-8.27	19.47	11.36
4	-21.55	0.0	6.21
5	-16.58	-11.29	-6.01
6	-20.44	-15.92	-18.89
7 (Apex, i.e. oldest)	-22.57	-17.52	-17.02

(Segment 3 was the first greenish segment; 4 was fully green.)

Experiments carried out with *Pelargonium* leaves of different ages gave generally similar results, and Series 3 (Table IV) represents one set of results which are in some respects informative. The leaves are arranged in order, those from the youngest nodes having the lowest number. These leaves usually showed hydrolysis of protein throughout, except in 3 per cent. glucose $\times$ 0.2 per cent. ammonium nitrate or in the series where asparagine of the same nitrogen content was substituted for ammonium nitrate. This series was done at the same time and on similar leaves to those used in the series preceding it in the table. While the general effects of organic sources of nitrogen will be left until a later paper, in this instance the high protein synthesis in the youngest leaves when asparagine is supplied, is of interest in suggesting that the lower figures in the corresponding ammonium nitrate cultures may be due to the injurious effects of ammonium ions.

In general, this series for *Pelargonium* illustrates the importance of suitable concentrations of nitrogen and glucose in this type of experiment.

The leaves floating on 6 per cent. glucose were quite flaccid.

It is of interest to note that plasmolysis in this case led to more extensive hydrolysis of protein, particularly in the youngest and older leaves. This is possibly an effect similar to the hydrolysis of starch observed in desiccated leaves and potato tissue.

TABLE IV

Effect of Varying Concentrations of Nitrogen and Glucose on the Percentage Gain of Protein by Leaves of Pelargonium zonale. Fresh-weight Basis.

(Duration 72 hours, half-leaf and cold alcohol methods.)

Glucose (%)	0.4	0.5	1.5	6.0	3.0	3.0	3.0
NH ₄ NO ₃ (%)	0.025	0.037	0.075	0.1	0.075	0.2	0.33 ¹
Node							
2	-9.6	-16.5	-20.0	-30.6	-14.5	1.4	32.2
3	-4.4	-16.5	-15.0	-16.4	-17.4	17.8	32.9
4	—	-24.1	-25.0	-7.6	-13.1	8.15	3.9
5	—	-15.5	-6.6	-24.3	-15.4	4.0	5.8
6	-7.1	-8.8	-12.6	-19.0	-16.3	1.3	-2.0
7	-10.5	-13.1	-13.2	—	—	-1.4	-1.3
8	—	-19.0	—	—	—	—	—

¹ Asparagine.

EFFECT OF LEAF AGE ON PROTEIN SYNTHESIS

The data shown in Tables II and III agree with a wide range of data obtained with daffodil leaf segments and these will be discussed more fully in subsequent papers, especially in relation to various other aspects of protein synthesis in leaves. In most of these experiments, the leaves were divided into four segments (1) basal, white and meristematic, (2) elongating zone, yellow to pale green, (3) younger mature, full green, (4) apical and oldest green part of leaf. The method of division has thus a physiological basis, although the separation of the zones can obviously not be very exact. A typical series, using the drying method of preparing the leaves, is given below. This method enables the water content of the experimental material to be determined, a feature of interest in relation to the very different nitrogen contents of the segments. It will be observed that the different proportions of nitrogen in the various segments are not wholly due to the varying proportions of dry weight present.

The series recorded in Table V is for leaves about three-quarters of full size. In such leaves, synthesis is observed only in the basal or meristematic segments. With earlier leaf stages, some protein synthesis may also occur in the elongating segments (cf. Table III).

These experiments agree consistently in showing that in daffodil leaf segments, the young (meristematic and vacuolating) tissues are the only ones

capable of protein synthesis. By the time the tissues have reached their full size and are fully green, the capacity for protein synthesis seems to have vanished, under these conditions. This is equally true of *Pelargonium* leaves. The second part of this investigation was therefore directed towards determining whether this feature was of general occurrence in isolated leaves.

TABLE V

Total and Protein N (mg. per 100 gm. Original Fresh Weight)—Narcissus Leaves

Segment	Water content (%)	Original		Experimental gain	
		Total N.	Protein N.	Total N.	Protein N.
1 (Base)	91.35	231.8	138.1	+68.5	+14.7
2	90.39	307.6	178.9	+56.8	-10.9
3	86.29	496.5	373.9	+100.1	-11.0
4	81.98	725.7	624.6	+79.8	-50.2

To make this survey as general as possible we examined a variety of structurally different leaf types including leaves of *Tropaeolum majus* (Sutton's Giant), and *Vicia faba* as annuals, *Coleus* sp., and a small-flowered *Helianthus* (*H. perennis*) as perennials, *Ligustrum vulgaris* as a woody plant, and *Iris pseud-acorus* as another monocotyledon.

The results of these experiments are given in Table VI and in the Appendix. In each case the leaves were floating on the nutrient medium for between fifty and seventy-two hours. The half-leaf method was used except for *Helianthus* and *Vicia faba*, where the twin-leaf or leaflet method was substituted. *Tropaeolum*, *Helianthus*, and *Vicia* leaves were dried. Privet leaves were extracted with water after etherization. *Coleus* and *Iris* leaves were extracted with cold alcohol. The full results and leaf-lengths, where available, are given in the Appendix. The data are on a fresh-weight basis for all except *Iris*, where the area basis was used. Nodes are numbered from the apex.

The results given in Table VI agree with the earlier ones in showing that protein synthesis under these conditions is a property of the younger leaves. The data show that rapid protein synthesis has usually ceased by the time the leaves are half grown (in size), while protein synthesis is absent in mature leaves or leaf segments and later tends to be replaced by hydrolysis. In drawing this conclusion we would emphasize the fact that in no case were the leaves used senescent or even approaching the senescent stage. They were, normally, in fact, young mature rather than old. The data for broad bean (plants with young fruits) include the oldest leaves used (though these were still perfectly green) and they show a general similarity between node 9 and nodes 19-20.

One possibility which must be considered is that the leaves were under abnormal conditions when floating on nutrient media in the dark. So far as could be ascertained by microscopic examination the tissues remained quite normal, and in particular the air spaces remained uninjected throughout the

experiments. The possibility that carbon dioxide might accumulate in toxic quantities exists or that oxygen deficiency might develop, but if so one would expect the youngest tissues to suffer most severely especially as their system of aeration appears to be less complete. Finally the results to be considered

TABLE VI

Percentage Increase in Protein Nitrogen in Leaves of Different Ages, Floated on 3% Glucose, 0.2% Ammonium Nitrate for 50-72 Hours.

Node.	Coleus.	Ligustrum.	Iris.
2	21.96	12.72	18.64 segment 1 (base)
3	8.60	8.50	3.81 " 2
4	7.28	-1.04	1.74 " 3
5	6.16	0.84	4.16 " 4
6	0.0	1.31	-17.93 " 5
7	0.5	—	-17.25 " 6 (apex)
8	—	-1.88	—
10-11	—	-2.21	—
	Helianthus.	Tropaeolum.	Vicia faba.
2	9.62		20.72
3	9.54	41.44	19.19
4	1.57		21.82
5	-1.17	30.16	15.67
6	1.10		—
7	—	10.41	—
8	—	8.92	—
9	—	-1.97	-14.65
10	—	1.43	—
12	—	-1.31	-13.33
14, 15	—	-6.89	-11.21
19, 20	—	—	-13.36

in the next section strongly suggest that the inability of the older leaves to form protein is due mainly to their physiological condition and not to any condition of experimental procedure.

It appears then that the growing leaf is capable of synthesizing protein while the old one is not. Roughly speaking, this change in the activity of the leaf comes about the time when the leaf becomes green; and fully mature leaves in these experiments always showed a marked decline or absence of protein synthesis. In essence, then, the normal leaf passes through two main stages—an earlier one when it is engaged in protein synthesis and a later one when it is photosynthetic. These two phases must undoubtedly overlap to some extent when the leaf is still growing but green, but their separation in time is real and this fact seems to be a strong argument against any theory of protein synthesis which may be based on its supposed resemblance to photosynthesis.

PROTEIN SYNTHESIS DURING THE FLOWERING AND FRUITING STAGES

By the time this survey was complete, although it included other results not given here, only one exception to the generalizations recorded was

observed. In this case, a second series of *Coleus* leaves later in the season showed considerable gains of protein in the older non-growing leaves. An examination of the data showed that the only peculiar features of this series were firstly, that the plants were now flowering, and secondly, that the older leaves contained abnormally low proportions of protein.

TABLE VII

Difference in Protein Synthesis and Nitrogen Content between Non-flowering and Flowering Stages of Coleus. (Half-leaf and Cold Alcohol Methods)

Nitrogen as mg. per 100 gm. of fresh weight.						
Non-flowering.			Flowering.			
Node.	Original protein N.	Final protein N.	Gain (%).	Original protein N.	Final protein N.	Gain (%).
2	214	261	22.0	204	253	24.0
3	221	240	8.6	179	194	8.4
4	206	221	7.3	142	161	13.4
5	200	213	6.2	104	121	16.36
6	194	194	0.0	95	114	20.0
7	201	202	0.5	—	—	—

Thus while the youngest leaves were essentially similar in protein content and in activity, the older leaves (e.g. node 6) in the flowering stage only contained half the quantity of protein, and also had a considerable capacity for protein synthesis. It appeared to be possible then, that flowering led to a considerable drain of nitrogen away from the leaves along with a revival of the capacity of the leaves to synthesize protein. Further results in this laboratory show that leaves of *Potamogeton perfoliatus* may lose to the flowering shoot at least 30 per cent. of their nitrogen. Both Gouwentak (1931) and Smirnow (1928) have observed that at the time of flowering leaves possess the smallest amounts of nitrogen recorded. Mason and Maskell's observations also afford evidence of movement of nitrogen from leaves of cotton plants in the flowering state, while Acharya and Sastra (1931) also point out that there is a general movement of all types of substances towards the developing flowers, so that the quantities of labile material elsewhere in the plant may diminish at this stage. It appears possible, then, that in some plants a large drain of nitrogen from the leaves may take place during flowering, and that as a result of this, protein synthesis in the older leaves may be again possible. It is clear that this conclusion is not of general application.

A very different set of facts was obtained from a short study of a double-flowered *Narcissus* (Sutton's Emperor), in which the bulbs were grown indoors in soil in boxes under approximately constant conditions of light and temperature. The boxes were illuminated by 100-watt 'daylight' lamps in such a manner that each row of plants received approximately equal radiation as measured by a sensitive photo-voltaic cell. The variation in light intensity at soil level was less than 3 per cent. of the mean and was checked at intervals. The temperature varied between 12 and 17° C. at different times during the

growth period, though these variations were the same for all plants and the temperature fluctuations were normally small. The average weight of the bulbs was 65 gm., and those used were selected for similarity of appearance and limited to bulbs between 60 and 70 gm. in weight. The leaves of these plants were shorter and more fleshy than those of the single-flowered form. They were very mucilaginous on cutting and did not appear to be quite so efficient in protein synthesis under experimental conditions. Four samples were taken, the last two on the same date representing leaves of different lengths and ages. Flowers were opening in the plants used for the fourth sample. The pertinent data from these collections are given below.

TABLE VIII
Protein Content and Gains by Narcissus Leaves (mg. per 100 gm. of Original Fresh Weight.)

Date.	Leaf length (cm.)	Half-leaf and Hot Alcohol Methods				
		Part of leaf.	Water content (%)	Soluble N.	Protein N.	Experimental gain in Protein N.
1 Feb. 5	7-8	{ Base	88.37	77.5	260.5	8.5*
		{ Apex	87.64	96.5	302.7	6.5
2 Feb. 19	8-12	{ Base	86.34	85	365	23.0*
		{ Apex	85.90	102	416	-10.0
3 Feb. 26	14-17	{ Base	89.39	82	243	32.0*
		{ Apex	88.19	91	326	-1.0
4 Feb. 26	23-26	{ Base	89.74	83	224	6.0
		{ Apex	88.90	80.5	255	-3.0
		{ Apex	87.90	90	332	-4.0

* Significant gains.

The table shows that the protein N (on the fresh-weight basis) of these leaves remains approximately constant until the stage represented by set 4, when flowers were ready to emerge. The higher protein content of set 2 is partly counterbalanced by the low water content of these samples. As the leaves in set 4 were more than three times the length of those in set 1, the total leaf protein in set 4 must have been of the order of three times that in set 1. The leaves, then, were gaining nitrogen all through the period when flowers were developing. The flower primordia are, of course, normally present in the bulb in this genus, so that the developmental cycle bears a resemblance to that of *Lupin* discussed below. In *Narcissus*, however, the oldest parts of the leaves have lost their capacity for protein synthesis at an early stage, and do not regain it. As the leaf appears to remain, as it were, saturated with nitrogen during the development of flowers—no intensification of protein synthesis at this stage can be detected. One other feature of the *Narcissus* leaf is the high nitrogen or protein content of the apical portion. The basal portion is normally white and semi-meristematic, but contrary to usual experience with meristematic tissues it contains less protein than does the mature apical region.

We have a large number of series dealing with *Narcissus* leaves, many of which will be discussed more fully in later papers. The main interest of these results in the present connexion is that for the whole leaf the protein N content remains approximately constant whether the leaves are collected when short or when flowers have already appeared. There are, therefore, no grounds for believing that any appreciable loss of nitrogen takes place in the early phases. Further, no gains in protein (under the stated experimental conditions) have been observed in leaves from plants with fully open or withered flowers.

When the flowers wither, the growth of the leaves ceases and their protein content normally falls. Another sign of evacuation, that of yellowing at the tip, becomes visible about this time.

It may be suggested that the maintenance of the nitrogen content of the *Narcissus* leaf during flower development may be a result of its organization. The basal meristematic region may control to a high degree the entry and exit of substances if, as is sometimes assumed, such tissues are less permeable than are mature cells. The role of the vascular strands running through this basal meristem may also repay attention. Although xylem vessels are well developed in this region at an early stage the phloem appears to develop later.

Data for a leguminous plant, lupin, are also available. In this case observations were made on young shoots from mature roots, from shoots with flowers and from fruiting plants, the twin leaflet method being used. All the material came from one set of well-established plants. In the case of the plants with flowers, from each leaf four similar leaflets were chosen, one of these being used for the original nitrogen content, one being placed in the usual sugar and ammonium nitrate solution, another in sugar and asparagine (of the same nitrogen content), and the last being left attached to the plant. The protein N of the attached leaves was estimated as mg. per 100 gm. of final fresh weight as, of course, the original fresh weight could not be measured.

The drift of events appears to be very different in lupin from that observed in other genera. The leaf protein content increases during flowering and declines during fruiting, especially in the youngest leaves. Further, only in the leaves from fruiting plants is there evidence of a significantly greater capacity for protein synthesis in the young leaves. Normally in young or flowering shoots of lupin, protein synthesis is most marked in mature or nearly mature leaves. (In these series, the three youngest nodes in each series are still growing.) The similarity of the data for young shoots and for plants with flowers suggested that the former might already possess flower initials. Subsequent examination suggests that flower initials are always present in mature lupin plants, and hence that the young shoots described here should properly be regarded as representing the beginning of the flowering stage. In short, it appears that a well established lupin plant is already in the flowering condition when it starts to grow in early summer. Lastly no obvious

TABLE IX

*Plants of Lupin (Lupinus?) (Protein as mg. per 100 gm. Fresh Weight)
Twin Leaf and Hot Alcohol Method*

Original protein N.				Gains in protein N (%) under experimental conditions.				
Node.	Young.	Flower- ing.	Fruit- ing.	Flowering.				
				Young.	NH ₄ NO ₃	Aspara- gine.	Attached leaf.	Fruit- ing.
2	442	683	550	14.85	5.93	15.37	1.17	3.59
3	618	—	664	12.14	—	—	—	2.08
4	756	875	498	14.90	17.83	13.48	-1.02	1.93
5	895	953	587	5.45	10.60	11.54	0.05	-0.12
6	822*	951	481	-2.28*	2.31	3.78	-1.89	0.95
7	—	1,015*	519	—	7.68*	6.40*	-5.52*	5.62
8	—	—	422*	—	—	—	—	4.60*

* Youngest leaves.

correlation exists in lupin leaves between the capacity for protein synthesis (under experimental conditions) and the protein content. The differences between lupin and the other plants examined are, in fact, so marked that we think that attention should be drawn to the undesirability of applying to other plants conclusions based on lupins.

CONCLUSIONS

The general conclusions to be drawn from these observations are that the capacity of leaves for protein synthesis under experimental conditions may depend upon their age, their protein content and upon unspecified factors associated with the physiological organization and state of the whole plant. Possibly the permeability of the cells to dissolved solutes in the experimental medium may also be a factor of importance. The three plants examined all show different developmental stages of nitrogen metabolism in relation to flowering. It appears, therefore, to be unwise to attempt to apply to other genera conclusions based on any one type of plant.

APPENDIX

Summary of Experimental Data

Daffodils.

I. Protein N (mg. per 100 sq. cm. of leaf area) before and after the experimental treatment (72 hours). Ammonium nitrate solution 0.075 per cent., glucose varying as shown. Half-leaf and cold alcohol methods. Segments of leaf arranged in order of position.

Glucose (%).		1.		3.		5.	
Segment.		Original.	Final.	Original.	Final.	Original.	Final.
1 (Base)		18.3	17.3	10.4	11.4	15.6	17.8
2		19.2	18.6	15.1	14.9	19.9	18.9
3		22.6	19.8	19.5	19.6	22.0	20.7
4		23.2	21.9	21.9	22.4	24.2	22.3
5		27.9	24.3	26.9	24.5	28.2	24.5
6 (Apex)		30.1	28.4	32.0	30.3	32.9	28.3

II. As above: Solution, glucose 3 per cent. plus ammonium nitrate.

Am. NO ₃ (%).	0.1		0.15		0.2	
Segment.	Original.	Final.	Original.	Final.	Original.	Final.
1 (Base)	11.7	16.4	9.2	12.2	10.1	13.3
2	10.6	11.9	8.9	13.4	9.4	11.8
3	14.5	13.2	11.3	13.5	13.2	14.7
4	18.1	14.2	15.6	15.5	14.5	15.4
5	19.3	16.1	18.6	16.5	18.3	17.2
6	22.5	17.9	20.1	16.9	21.7	17.6
7 (Apex)	25.7	19.9	23.4	19.3	23.5	19.5

Pelargonium zonale.

Protein N (mg. per 100 gm. original fresh weight) with varying amounts of nitrogen and glucose in medium, 72 hours. Half-leaf and cold alcohol methods.

Glucose (%).	0.4.		0.55.		1.5.		6.0.	
Am. NO ₃ (%).	0.25.		0.37.		0.75		0.1.	
Node.	Original.	Final.	Original.	Final.	Original.	Final.	Original.	Final.
2	483	437	738	616	681	545	725	503
3	478	457	491	410	506	430	444	371
4			588	446	416	312	410	379
5			432	365	365	341	371	281
6	311	289	318	290	318	278	348	282
7	325	290	312	271	287	249		
8			342	277				

Glucose 3 (%).

Am. NO ₃ (%)	0.075		0.2		0.33 (asparagine).	
Node.	Original.	Final.	Original.	Final.	Original.	Final.
2 (Apex)	603	515	721	731	702	928
3	425	351	495	583	519	690
4	358	311	405	438	414	430
5	312	264	375	380	395	387
6	301	252	373	388	325	344
7			355	350	382	377

Iris Pseudacorus (non-flowering).

Protein N (mg. per 100 sq. cm. of leaf area).

Standard solution, 72 hours. Half-leaf and cold alcohol methods.

Segment	1 (Base)	2	3	4	5	6 (Apex)
Original	118	105	115	168	184	193
Final	140	109	117	175	151	179

Tropaeolum majus.

Protein N (mg. per 100 gm. original fresh weight).

Standard solution, 72 hours. Half-leaf and drying methods.

Nodes	2, 3, 4	5, 6	7	8	9	10	12	14, 15
Average leaf length (cm.)	0.5-0.8	2-3.5	4.3	5.6	8.4	10.1	10.0	11.5
Original	999	988	951	863	810	698	612	566
Final	1,413	1,286	1,050	940	794	708	604	527

Ligustrum vulgare.

Protein N (mg. per 100 gm. of original fresh weight).

Standard solution, 72 hours. Twin leaf and water extraction methods.

Nodes	2	3	4	5	6	8	10-11
Average leaf length (cm.)	2.2	3.6	4.4	5.9	6.7	7.3	7.4
Original	656.5	595.5	607.6	624.2	624.4	595.4	502.4
Final	740	646.1	601.5	629.5	632.6	584.2	491.3

Helianthus perennis (vegetative) and *Vicia faba* (fruiting).

Protein and soluble N (mg. per 100 gm. of original fresh weight). Standard solution.

Helianthus 50 hours, twin leaf and drying methods. Average leaf lengths in cm.*Vicia* 60 hours, twin leaflet and drying method.

Node.	Helianthus.			Vicia.			
	Original.	Experimental.		Original.		Experimental.	
	Length.	Prot. N.	Prot. N.	Prot. N.	Sol. N.	Prot. N.	Sol. N.
2	2.7	967	1,060	620	257	748	434
3	6.5	828	907				
4	10.5	698	709	676	271	806	491
5	10.8	426	421	621	181	756	379
6	7.5	182	184	562	174	650	359
9	—	—	—	720	145	615	366
12	—	—	—	666	129	577	338
14-15	—	—	—	507	131	451	309
19-20	—	—	—	311	81	269	232

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Cytology and Genetics of Some Indian Wheats

II. The Cytology of Some Indian Wheats¹

BY

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With Plates XIII to XV and two Figures in the Text

INTRODUCTION

THE cultivated species of *Triticum* can be divided into three groups, based on the number of their chromosomes. The Einkorn group comprising *T. monococcum* is characterized by seven pairs of chromosomes; the Emmer group comprising *T. dicoccum*, *T. durum*, *T. polonicum*, and *T. turgidum* has fourteen pairs of chromosomes; and the Vulgare group comprising *T. vulgare*, *T. spelta*, and *T. compactum* has twenty-one pairs of chromosomes.

The cytological work on the genus *Triticum* dates back to the year 1893 (cited in Percival, 1921), when Overton reported eight to be the haploid chromosome number of *T. vulgare*. His work was followed by that of Körnicke (1896), Dudley (1908), Nakao (1911), and Bally (1912). They all reported the same as Overton, and some of them claim to have seen even sixteen chromosomes in the somatic cells, but the later work showed all these results to be quite unreliable.

It was not until 1918 that the exact somatic counts of the chromosomes for the different species of wheat were published by Sakamura, who working with root-tips, found the somatic numbers to be fourteen, twenty-eight, and forty-two respectively, as stated above. This work was confirmed by Kihara (1919) and from meiotic counts by Sax (1922), and Watkins (1924).

Sax (1922), Thompson (1925), Watkins (1924, 1925), and others worked out the cytology of partially sterile interspecific wheat hybrids, particularly the cytological behaviour of the pentaploid hybrids obtained by crossing 28-chromosome wheats with the 42-chromosome ones. They observed that on crossing any of the emmers which are characterized by twenty-eight somatic chromosomes with any of the Vulgares which are characterized by forty-two somatic chromosomes, an F_1 plant with thirty-five chromosomes in its somatic cells is obtained. In meiosis of the F_1 the fourteen pairs of chromosomes behave normally, whereas the seven univalents show irregularity, so that the resulting gametes contain all numbers ranging from fourteen to twenty-one chromosomes. The nearer the number of chromosomes

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approaches fourteen or twenty-one, the more viable are the gametes, and the segregates with intermediate numbers are eliminated due to sterility. The partially sterile hybrids possessing twenty-one chromosomes resemble more the Vulgare and those possessing twenty-eight the Emmer parents respectively. Actually, however, in later generations segregates possessing either twenty-eight or forty-two chromosomes are obtained in a fertile homozygous condition.

In crosses between the first and the second group, Sax (1927) observed that in the first meiotic division the seven paired chromosomes divide normally, whereas the seven unpaired usually pass to one pole or the other without dividing. In the second division all the chromosomes apparently divide normally, so that the resulting microspores presumably receive from seven to fourteen chromosomes. But according to Thompson (1931) in a similar cross the seven singles lag in the first reduction division, but ultimately divide and join the bivalents. In the second division they pass to the poles without dividing, like the seven univalents of the Emmer $\times$ Vulgare crosses.

Besides the cytological studies of the interspecific hybrids, the cytology of the intergeneric hybrids, including the amphidiploids with double the number of chromosomes, between different species of Aegilops, Triticum, Secale, and Agropyron have been made by several investigators, and natural hybrids in some of these have frequently been observed. It was in fact these natural hybrids that brought into prominence the genus Aegilops with regard to the origin of soft wheats.

The occurrence of haploids in Triticum has also been reported on several occasions, as for instance Gaines and Aase (1926), Katayama (1934), Yamasaki (1936), and Smith (1936). Huskins and Hearne (1933) studied the meiosis in an asynaptic oat and wheat.

Thus during the past nineteen years or so the wheat cytologists throughout the world have made a thorough survey of the various cytogenetical problems concerning this important genus. The contributions of Sakamura, Kihara, and Kagawa of Japan, Gaines and Aase and Sax of U.S.A., Thompson in Canada, Percival and Watkins of England, de Mol of Holland, Tschermak and Bleier of Austria, Nikolaeva of Russia, Malinowski of Poland, and Stolz of Germany towards this subject stand pre-eminent among all the rest, and it seems quite obvious that nearly all the phases of cytology in this genus have received at least some attention; but as regards the satellites, which are now forming a very important feature in the morphology of plant and animal chromosomes, and their relation to the nucleolus, the genus Triticum has been totally neglected. It was with this object—and moreover the study of the possible cytological cause of the deterioration of 'Sherbati' hybrids necessitated the comparison of the morphology of the chromosomes of these hybrids with their parent—that the present work, which deals mainly with the morphology of the wheat chromosomes, their constrictions, satellites, and

the relation of the satellites to the nucleolus both in the tetraploid as well as the hexaploid wheats, was taken up. A reference to the origin of *T. vulgare* and the cytological cause for the deterioration of 'Sherbati' hybrids is also made.

MATERIAL AND METHODS

The cytological studies dealt with in the present paper cover the following wheats from the Department of Agriculture, C.P. and Berar:

1. *T. dicoccum* var. *Indicum* Bhatia, popularly known as 'Khapli' C.P.
2. *T. vulgare* var. *albidum* A1., departmental wheat Ao88 popularly known as 'Mudya',
3. A112, A113, and A115, which are popularly known as 'Sherbati' hybrids, are the three sister strains of the hybrid between *Vulgare* and *Khapli* Emmer, in about their twenty-third generation. These three strains appear to have merged into one another completely, and as already pointed out in the Report on Demonstration Work, Northern Circle (Department of Agriculture C.P. and Berar) year 1926-7, there seems to be no morphological difference between them, and from all points of view they can be treated as one.

The seeds of these varieties were sown in pots in the years 1935 and 1936 in greenhouses at the Courtauld Genetical Laboratory, Regent's Park, London. The sowing in 1935 was done during November, and that of 1936 during January, February, and middle of March. Those sown during November 1935 did not do well, and most of them died. The survivors lingered on miserably and came to flower in the month of April, along with the others sown in January 1936, which also remained very poor as regards their vigour. The rest of the lots sown in February and March were quite vigorous and normal in their growth, and their flowering lasted from May to the middle of June 1936. From all these experiments it was concluded that the best time for wheat-sowing in London is from the third week of February up to the first week of March.

Along with the plants grown in greenhouses, there were others grown outside in the open in pots. They were sown in lots from the middle of February up to the middle of March. They were the most vigorous of the whole lot, but came into flower later than the corresponding ones sown inside the greenhouses. It may be mentioned here that anybody working in London on wheats from the genetical point of view should be very careful about damage by birds and mice. It is necessary that precautions be taken from the germinating to the fruiting stages.

In order to study the morphology of the chromosomes in the somatic cells, the method described by Kagawa (1929) was followed. The kernels were germinated in small Petri dishes on wet filter paper. When the seminal roots were about 1-2 cm. the kernels were immersed in 0.4 per cent. aqueous solution of chloral hydrate for one hour, and this was followed by washing

in running water for the same length of time. After keeping these kernels in a moist space for about an hour or so, the root-tips were fixed in Benda's solution and La Cour's 2BE, omitting acetic acid in both cases. Other fixatives such as Navashin's and Allen's modifications of Bouin's were also tried, but La Cour's 2BE gave decidedly superior results.

Paraffin sections were cut 15–20 μ in thickness and stained with Newton's iodine gentian-violet technique. The best time for fixing the root-tips was found to be on bright sunny days between 11.30 a.m. and 1.30 p.m. when the division in the growing root is very vigorous. For studying meiosis, after removing the glumes, the flowers were trimmed a little at the apex to allow an immediate contact of the fixing fluid with the anthers, or the anthers were taken out and fixed separately. In either case a preliminary examination to see the stage of division was made by the iron-aceto-carmin method. Before fixing the flowers or the anthers they were dipped for a few seconds in Carnoy's solution, and then fixed in Navashin's Fluid (Maeda, 1930), La Cour's 2BE, and Medium Flemming. Navashin's Fluid after pre-treatment with Carnoy gave decidedly superior results. Both for fixing root-tips and flower buds an exhaust pump was always used. With experience, simply by looking at the young ear one is able to say whether it will show any division or not, or whether it is too early for division, or has passed the division stage.

In London during the months of April, May, and June the writer has found the anthers in the division stage between 11 a.m. and 2 p.m. The different flowers of the same spikelet may show all stages of division, and it was observed that the anthers of the same flower ordinarily are in the same stage. It may also be said in general, that if the oldest flowers of a spikelet show young pollen grains, the next younger flowers will show the second meiotic division and tetrads, the next younger will show stages of the first meiotic division, still younger ones the early prophase stages, and the youngest will not show any division at all. The spikelets in the middle of the ear are the first to show division, and those at the top or base lag a day or two behind the central ones in corresponding stages.

It is necessary to lay special stress on the importance of a careful de-staining of the nucleolus, the control of which is acquired gradually with experience, in studying its relation with the chromosomes in meiosis.

SOMATIC DIVISION

In somatic mitosis the morphology of the metaphasic chromosomes only has been studied. The morphology of the chromosomes of *Triticum* was first studied by Sakamura (1920) when he reported some chromosomes having two constrictions. Kagawa (1929) made a very exhaustive study of this subject in the chromosomes of *Triticum* and *Aegilops*. Camara (1935) and Ellenhorn (1935) made a short survey of the morphology of chromosomes in *T. monococcum*. All these workers failed to show the presence of satellites in any of the wheat chromosomes.

The credit of reporting the satellites in *T. monococcum* goes to Smith (1936), who has shown them present on two pairs of chromosomes. According to him the larger satellite, which is on the shorter chromosome, is less commonly observed than the other, apparently because it is not set apart so far from the main body of the chromosome. The longer chromosome with a satellite was associated with the nucleolus.

The satellites of *T. vulgare* and *T. dicoccum* were also observed by the writer in 1935, and as the present work has taken two years for its completion it has caused a delay in their publication.

By the action of chloral hydrate the constrictions other than the attachment constriction become clear, but they are not observable in all the cells and sometimes a cell may show them, whereas in the cell next to it in the same section they may not show. As already expressed by Kagawa (1929), the degree of ease with which the constrictions are fixed clearly, has probably some bearing upon the internal and external condition of the cell in connexion with the methods of fixing. Moreover the writer has observed that all the chromosomes of the same cell do not always show all the constrictions simultaneously, so one rarely gets a cell showing clearly their details in every chromosome. For that reason, in the present study the selection of the cells has fallen on very few which showed simultaneously all the constrictions.

Morphology of the chromosomes of T. dicoccum.

Text-figs 1A and 1B show the chromosomes from two different cells of *T. dicoccum*. The difference in the length and size of the chromosomes of the two cells is due to the action of fixatives. The chromosomes of Text-fig. 1A, which is a preparation from 2BE, are longer than those of Text-fig. 1B, a preparation from Benda fixative. In both cases the morphology of all the chromosomes is quite clear, and the homologous pairs, as shown in the figures, can easily be recognized.

Of the four satellited chromosomes (pairs 1 and 2) the two chromosomes composing pair No. 1 are tandem satellited chromosomes, that is, the morphology of pair No. 1 is different from that of No. 2. Of the remaining twenty-four chromosomes, twenty-two chromosomes have only one constriction each and the remaining pair (No. 10) have two constrictions each. As shown in the diagram, all the twenty-eight chromosomes fall into fourteen pairs and no two pairs are alike in the position of the constriction, showing clearly that the chromosome set of *T. dicoccum*, a tetraploid species, does not present the duplication of any diploid species, but is made up of two different sets which have already been designated as sets A and B by various workers, and the same has been expressed by Kagawa (1929). Contrary to Kagawa's observations, where he classified ten chromosome types in this species, the writer has observed fourteen chromosome types, that is, no two pairs of chromosomes are identical.

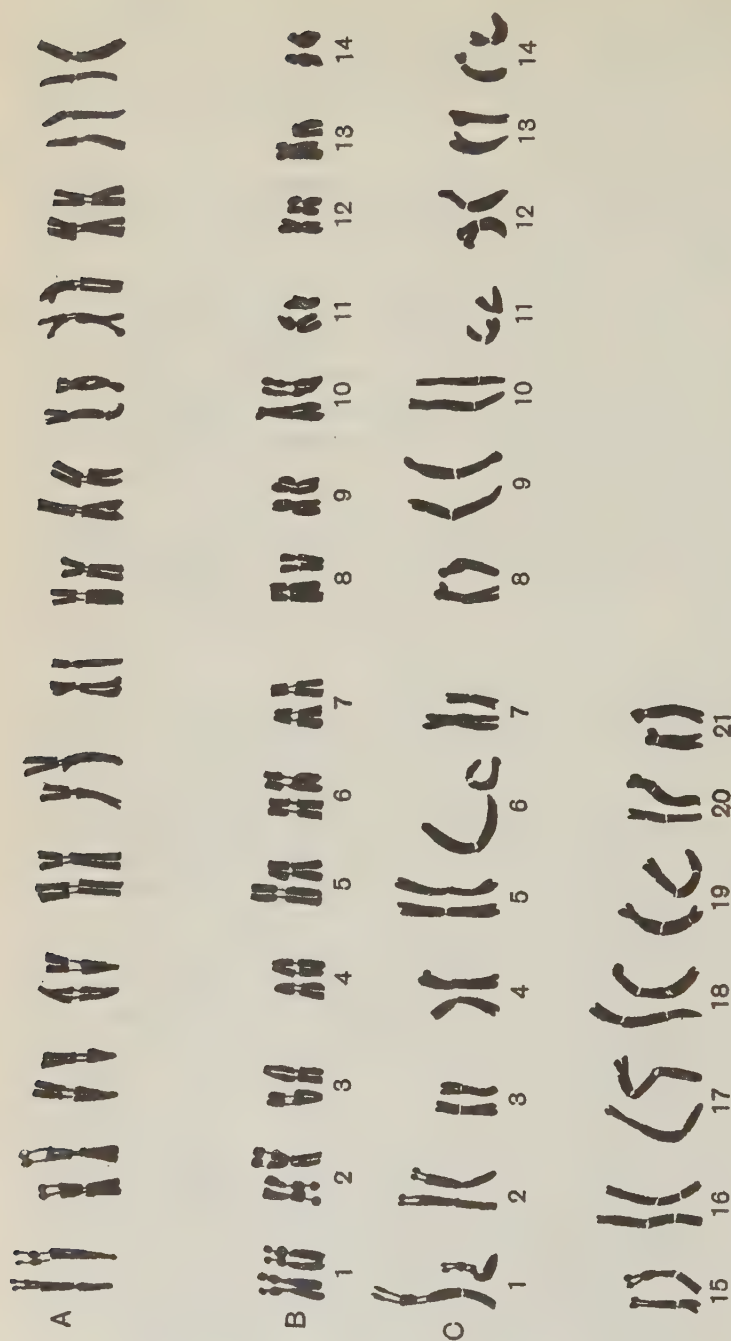


FIG. 1.

TEXT-FIGS. 1 and 2. Fig. 1A and B. The morphology of the chromosomes of *T. dicoccum* 'Khapli Emmer' in fourteen pairs. Pairs number 1 and 2 are satellited, pair No. 1 in both cases being tandem satellited. C. The morphology of chromosomes of *T. vulgare* in twenty-one pairs. Pairs Nos. 1, 2, and 15 are satellited, pair No. 1 being tandem satellited. Fig. 2A, B, and C. Morphology of chromosomes of the hybrids *T. vulgare* × *T. dicoccum*. Pairs nos. 1, 2, and 15 in all cases are satellited, pair No. 1 being tandem satellited.

All figures were drawn at the table level with the aid of a camera lucida. A 2 mm. Zeiss apochromatic, 1.3 aperture, and Zeiss ocular × 20 were employed for all drawings. The magnification of all the diagrams is 2900.

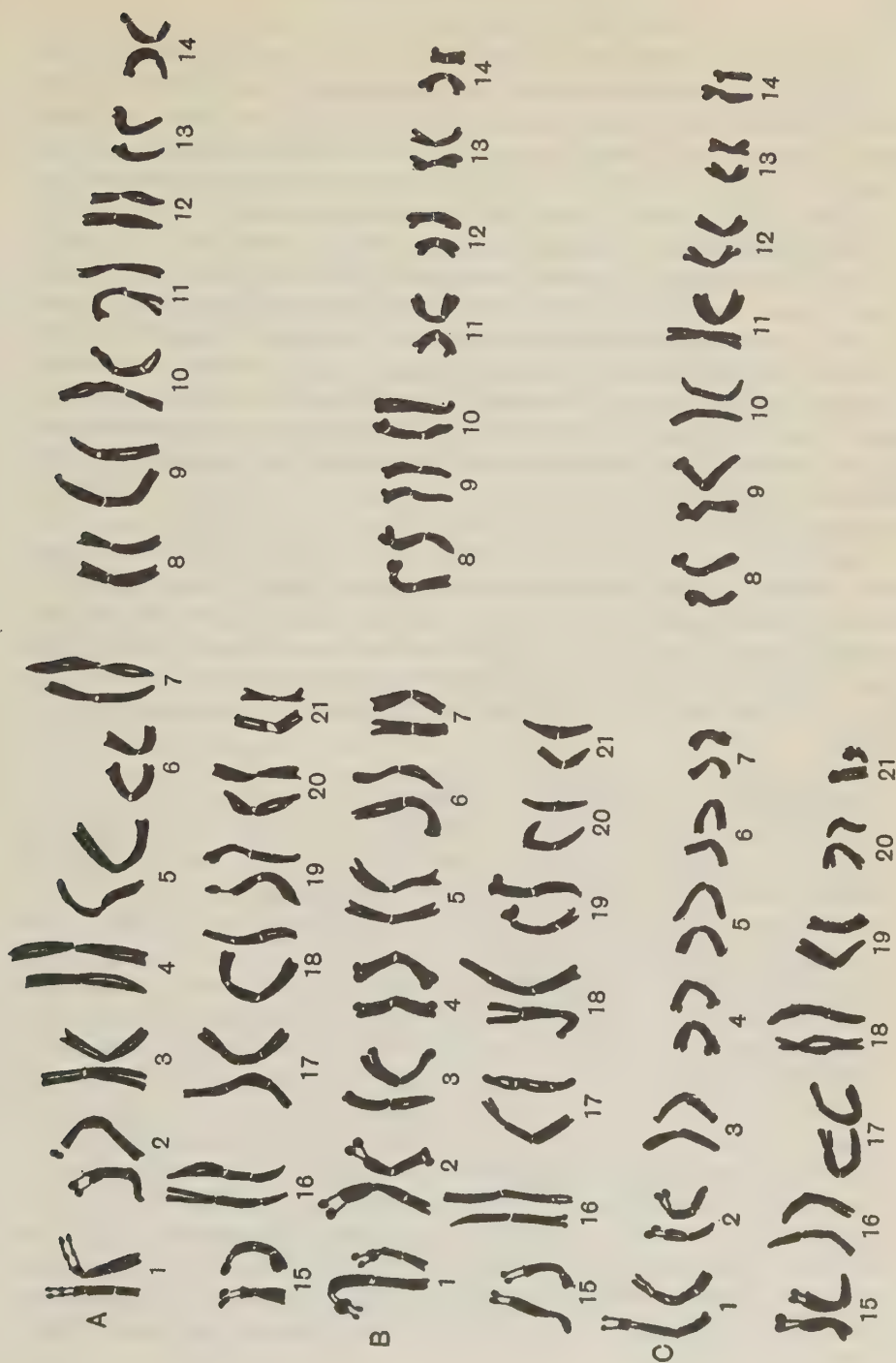


FIG. 2.

Morphology of chromosomes of T. vulgare.

Text-fig. 1C shows the morphology of the chromosomes from a single cell of *T. vulgare*. There are six satellited chromosomes, as shown in pairs Nos. 1, 2, and 15, the pair No. 1 being tandem satellited. Of the rest there are twelve pairs (Nos. 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14, and 21) with only one constriction, whereas three pairs (Nos. 10, 18, and 20) have two constrictions and the remaining pairs (Nos. 16, 17 and 19) three constrictions each.

The pairs from Nos. 1 to 14 have been shown to correspond to the pairs from Nos. 1 to 14 in *T. dicoccum*, leaving in *T. vulgare* the chromosome pairs from fifteen to twenty-one. On the basis that *T. vulgare* has originated from a cross between the Emmer and a species of Aegilops, it appears that the twenty-eight chromosomes comprising pairs 1 to 14 have been contributed from the Emmer parent and the remaining fourteen chromosomes comprising pairs 15 to 21, which are clearly missing in *T. dicoccum*, have been contributed from the other parent. In order to verify the truth of this statement, the study of the morphology of the chromosomes of Aegilops and allied genera is highly desirable.

From the morphology of chromosomes of *T. vulgare* it is evident that no two pairs of chromosomes are identical, showing clearly that the forty-two somatic chromosomes of *T. vulgare*, 'Mudya', a hexaploid species, do not present the triple state of the somatic chromosomes set of any of the diploid species, as already expressed by Kagawa (1929); but that the chromosomes represent three different sets which have already been designated as A, B, and C.

The morphology of chromosomes T. vulgare × T. dicoccum.

Text-figs 2A, B, and C show the morphology of the chromosomes of hybrids *T. vulgare* × *T. dicoccum*. Fig. 2A is from the strain A 115, whereas Figs. 2B and 2C are from the same preparation of the strain A 113. In *T. vulgare* twelve pairs of chromosomes with one constriction each were identified, but here in Fig. 2A, thirteen pairs (pairs Nos. 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14, 20, and 21) are identified. Three pairs (Nos. 10, 17, and 18) have two constrictions and pairs Nos. 16 and 19 three. One of the chromosomes of the pair No. 19 appeared to be satellited in addition to the three pairs already mentioned (Nos. 1, 2, and 15). In Fig. 2B there were three pairs (Nos. 1, 2, and 15) of satellited chromosomes as usual, twelve pairs (Nos. 3, 4, 5, 6, 7, 9, 11, 12, 13, 14, 20, and 21) of chromosomes with only one constriction, four pairs (Nos. 8, 10, 18, and 19) with two constrictions, and two pairs (Nos. 16 and 17) with three constrictions in their chromosomes. In Fig. 2C there are as usual three pairs (Nos. 1, 2, and 15) of satellited chromosomes, and twelve pairs (Nos. 3, 4, 5, 6, 7, 9, 11, 12, 13, 14, 20, and 21) having one constriction, three pairs (Nos. 8, 10, and 19) with two constrictions, and No. 18 with three constrictions, whereas the pairs Nos. 16 and 17 have either two or three constrictions each.

Comparing thus the morphology of chromosomes of the hybrid with that of the parent *T. vulgare*, it is evident that in those hybrids shuffling of the parental chromosomes in meiosis has taken place, as explained elsewhere. The presence of satellites in the tetraploid and hexaploid wheats is here described for the first time. Satellites were discovered by S. Navashin (1912) on the chromosomes of *Galtonia*. Later Sorokin (1924), Senjaninova (1926), and de Mol (1927) observed that the number of nucleoli corresponded with the number of satellited chromosomes. Taylor (1925, *a*, *b*, and *c*) showed satellite chromosomes in *Crepis setosa*, *C. capillaris*, *Aloe saponaria*, and *Gasteria*. Later (1926) he observed minor differences between satellites in a given cell as well as between the corresponding pairs in different roots, and emphasized the effect of the action of fixing fluids of various types upon the prominence of the various features. He concluded that the phenomenon of satellites and of chromosome constrictions shows that these features are important hereditary morphological characters of the chromosomes. At the same time he observed in *Fritillaria imperialis* two pairs of metaphase chromosomes having distal satellites of different sizes, in *Alstroemeria brasiliensis*, one metaphase pair bearing small proximal satellites, and in one plant of *Allium cepa*, a single 'J'-shaped element bearing tandem satellites on the shorter arm. Kaufmann (1926) reported satellites in the anaphase cells of *Tradescantia pilosa* as most delicate terminal appendages simply embedded in the apex of a conical projection of the chromatic material.

M. Navashin (1925) determined in *Crepis* species two types of chromosomes; type A, including all the two-armed chromosomes and type B including the satellite chromosomes represented by only one form. In 1926, from his studies of the trabrants of *Crepis dioscoridis*, he expressed the idea that the first step in the formation of species may be seen in the very small changes which appear in the dimensions of the trabrants of the D chromosome in *Crepis* species. According to S. Navashin (1927) the individuals of *Galtonia candicans* belong to two different species, as regards their chromosomes, the symmetrical plants having trabrants of the same size and the asymmetrical plants one small and one big trabant. He also obtained one bulb, the root of which showed an intermediate condition as regards the size of the trabrants. According to him the male and female plants of *Najas major* showed no difference in their satellites, both belonging to the symmetrical race having equal sized trabrants.

M. Navashin (1927) observed that through hybridization certain changes were brought about in certain chromosomes. For instance, in the hybrid *C. capillaris* × *C. tectorum* the most characteristic part, the trabant of the D chromosome of *tectorum*, which under normal conditions is always present, was missing. The same was observed in the cases of *C. capillaris* × *C. parviflora* and *C. rubra* × *C. foetida*. This change was concerned only with one of the two homologous chromosomes while the other remained unaltered. From these experiments he was led to conclude that these changes appear to be of

equal value to the changes or transformation of the hereditary units. Polymorphism with regard to the presence or absence of one or both satellites of a particular pair of chromosomes was found by Emme (1925) in some species of *Hordeum*. According to Medwedewa (1930) there are three races of *Crepis dioscoridis*, one with large satellites, one with small satellites, and a third heterozygous which gives offspring of the three types in the ratio of 1 : 2 : 1.

M. Navashin (1934) from his experiments came to the conclusion that in all the investigated individuals of the interspecific hybrid *C. capillaris* × *C. tectorum*, the D chromosome of *Tectorum*, which is characterized under normal conditions by the presence of a satellite, invariably appears without the satellite, while the satellited *Capillaris* chromosome remains intact. In the hybrid *C. capillaris* × *C. neglecta* all the chromosomes of *Neglecta* appear markedly shortened and thickened as compared with the normal condition, and in addition the satellited chromosome in *Neglecta* appears without a satellite. In a third case, in the cross *C. capillaris* × *C. parviflora*, both parental chromosome sets suffer striking alternations, viz. the D chromosome of *C. capillaris* loses its satellite and all the chromosomes of *C. parviflora* appear very much shorter and thicker than they are in the species. He further thinks that the foreign sets somehow mould each other, and he expressed this idea by the term 'amphiplasty', and the result of amphiplastic action was shown to be independent of the direction in which the cross was made. The situation was observed to be the same in all the allotripleids, in balanced amphidiploids, and the highest grades of polyploidy showed the same. It was also found that the chromosomes suffering amphiplastic changes under hybrid conditions immediately recover their normal shape as soon as the chromosomes of the other species are eliminated in the segregates.

Fernandes (1935), studying the satellites of *Narcissus reflexus* and *N. triandus*, found that both these species are very polymorphic from the point of view of the shape of their satellites, their presence or absence. He claims to have observed all degrees of transition from forms whose size is nearly equal to half a chromosome arm to a simple filament and even the absence of the filament. He denies the existence of symmetrical and asymmetrical races from the point of view of the satellites in *Narcissus*. He is of opinion that the size of a satellite is not constant in different individuals and that it varies greatly in the cells of the same individual.

In a later publication Fernandes (1936) does not consider the nucleolar chromosomes and the satellite chromosomes to be synonymous, and the filament of the satellite he thinks is not indispensable to the formation of the nucleolus. He has divided satellites into two subheads:

1. Hetero-chromatin satellites having all the characters of hetero-chromatin, which remain attached to the nucleolus during telophase, interphase, and prophase.

2. Eu-chromatin satellites—having the characters of eu-chromatin, since they undergo transformations during the telophases and prophases and are

not visible at the surface of the nucleolus during interphase and the first stages of prophase. The position of the point of greatest activity of the nucleolar forming region is not always the same, but it varies with the shape of the satellite in the metaphase chromosomes.

In the present material the writer has observed minor differences in the size and shape of satellites in the same species, as observed by Taylor, but from the present work it is not possible to say anything in the way of confirmation or contradiction of the work of Navashin or of Fernandes, though the importance of satellites as hereditary morphological characters of chromosomes is quite obvious. It is probable, however, that Fernandes has underrated the wide variation in apparent satellite-size which can occur as the result of inadequate control of the staining. It is suggested that the larger satellites observed by Smith in *T. monococcum*, which are on the shorter chromosome and less commonly observed, and which are not associated with the nucleolus, may be a case of eu-chromatin satellite as described above by Fernandes.

Origin of Triticum vulgare.

The problem of the origin of *T. vulgare* has received considerable attention from various workers. Percival (1921) thinks that *T. vulgare* is a vast collection of mutants and hybrids which he regards as having originated from crossings of *T. dicoccoides* or *T. dicoccum* with one or two species of *Aegilops* (*A. cylindrica* or *ovata*). From his experiments in 1930 he concluded that the bread wheats have arisen by hybridization, and that one of the parents is *A. cylindrica*. Tschermak (cited by Senjaninova-Korchagina (1932) attributes the origin of soft wheats to crosses with wild *Aegilops*. A theory has been held by McFadden (1930) that modern cultivated forms of wheat originated from inter-crossing between certain wild wheat-like grasses, such as wild rye, wild emmer, wild einkorn, and some one or more of the various species of *Aegilops*.

While all these theories are held, still according to Senjaninova-Korchagina (1932) there is no direct proof concerning the origin of soft wheats, and the question remains unsettled. The same author holds that, judging from the number of successful crosses between wheat and *Aegilops*, which is not great, and the progeny of which is nearly always sterile, the obtaining of octoploid *Aegilotriticum* instead of an expected hexaploid is no direct proof of Percival's hypothesis, and it only accentuates the deep heterogeneity of the parental sets. Moreover, the conjugation of chromosomes in *Aegilops*-wheat and in wheat hybrids with distinct heterogeneity of the chromosomes, according to the same author, is a temporary and accidental phenomenon little indicative of the relationship of the chromosomes. It may be mentioned here that Sapehin (1933) thinks that the data as to the number of bivalents cannot serve immediately as an index either of the homology of chromosomes or of the chromosomal relationship of the species. The conjugation or non-conjugation of the chromosomes shows only the behaviour of the genes of conjugation

in the environmental surroundings. Percival (1936) holds that the type of union among univalents at the metaphase of the heterotypic division may be used as a measure of homology of the conjugating chromosomes.

Bates (1931) concluded that bread wheats appear to have been derived from crosses between tetraploid and diploid species of wheat, forming triploid sterile hybrids in which chromosome doubling then gave rise to fertile hexaploid forms. 'While the details therefore remain uncertain, there is evidence for concluding that the origin of the hexaploid wheats has involved inter-specific and intergeneric crossing, with allopolyploidy and probably also autopolyploidy, combined with the occurrence of numerous parallel unit mutations.' The successful crossings of three species of *Agropyron* with wheat, according to Hurst (1937) shows a very close affinity to the former with the latter; and Vakar (1935) from his experiments concluded that *Agropyron elongatum* is allied not only to wheat but also to rye and to *Aegilops*. The near relationship of wheat with *Secale* is also evident from the obtaining of amphidiploid Triticale.

Judging from all these facts, and also since the hybrids between *Aegilops* and *Triticum* have been obtained under natural conditions, the writer is inclined to think that *Aegilops* appears to have played a considerable part in the origin of bread wheat, but not all, and that the working out of the morphology of the chromosomes of wheat and the allied genera will open for us a more reliable and accessible way to the synthesis of soft wheats, and as already suggested by Percival (1930) will also lead us to the analysis of the relationships existing between species which can be crossed.

MICROSPOROGENESIS

While dealing with the microsporogenesis in wheat, special stress will be laid on the relation of the satellite-chromosomes to the nucleolus both in tetraploid and hexaploid wheats.

Resting nuclei of the pollen mother-cells. The pollen mother-cells (Pl. XIII, Fig. 8) after the last premeiotic division are distinctly polygonal in section like those described in *Lathyrus odoratus* by Latter (1926), in *Oenothera* by Sheffield (1927) and in rice by Nandi (1937). They are situated in two rows in the loculus, and could be described as loosely packed, and the tapetal cells are always uninucleate at this time. The resting nucleus (Pl. XIII, Fig. 8) of each pollen mother-cell is more or less spherical in shape, and its granular reticulum always takes on a very deep stain with gentian violet. It contains from one to four nucleoli (Pl. XIII, Fig. 8) in the tetraploid wheat, and from one to six in the hexaploid wheat. The number six in the hexaploid is very rare, and it has only been seen once when all the six nucleoli were observed fusing in pairs (Pl. XIII, Fig. 18). Ordinarily the presence of one or two nucleoli in the tetraploid, and one, two, or three in the hexaploid is more common. More than one nucleolus in the resting stage of the pollen mother-cell has also been recorded by Latter (1926) in *Lathyrus odoratus*, and by Fikry (1930) in the

last premeiotic division of *Rumex scutatus*, in the pollen mother-cells of rice by Hedayetullah (1933), and many others.

Of the four nucleoli of the tetraploid wheat, two are bigger and two smaller (Pl. XIII, Fig. 8), whereas of the six nucleoli of the hexaploid wheat four are bigger and two are smaller (Pl. XIII, Fig. 18), as also observed in the root-tip cells (Pl. XIV, Figs. 31 and 32). They take a very deep stain with gentian violet, but if destained they lose their stain more rapidly than the corresponding cloudy chromatin reticulum, enclosed in which their characteristic moon-like appearance is very remarkable. They are nearly always spherical in shape, they may occupy any position in the nucleus, and are vacuolate from the earliest stages. In the present material a single large vacuole, which attains a considerable size in some of the single complex nucleoli in leptonema stage, or several vacuoles in nearly all stages of meiosis have been observed. In some resting stages particularly in the somatic cells (Pl. XIV, Fig. 31) no vacuole was seen. Vacuolation of the nucleoli has been reported by several investigators in different plants. Wager (1904) observed it of general occurrence in the root-tip cells of *Phaseolus*, and according to Latter (1926) the honeycomb appearance of the central part of the nucleolus of *Lathyrus odoratus* is due to the presence of numerous small vacuoles. These attain in many cases a considerable size in the root tip-cells of *Hyacinthus* according to de Mol (1926 *b*), who also observed that their number varies sometimes in different nucleoli, and in the variety *La Grandesse* it was striking that many nucleoli occurred with one large vacuole in the centre. A single large central vacuole was also frequently observed by Sheffield (1927) in *Oenothera*, and in *Oryza sativa* it attains a comparatively very large size according to Selim (1930).

Gates and Latter (1927) are of opinion that the vacuolate condition of the nucleolus during thread formation of *Lathraea* suggests loss of nucleolar material and its utilization in chromatin formation. Fikry (1930) holds that the vacuoles of the nucleolus in the pollen mother-cells of *Rumex scutatus* become more apparent in the prophase, and he is inclined to believe that in this case the vacuolation of the nucleolus is due to treatment.

Zirkle (1928), from his experiments in *Zea mays*, concluded that a 2 per cent. solution of acetic acid fixes the nucleoli as large vacuolate bodies, which do not retain the hematoxylin stain. A mixture of 4 per cent. formalin and 2 per cent. acetic acid fixes them as a solid densely staining body, but the same author (1931) observed that in the living cambial cells of *Pinus* the nucleoli contain small droplets of a less refractive substance, and consequently appear vacuolate. It may be mentioned here that the vacuolate condition of the nucleoli in *Zea mays* was particularly noticeable according to Fisk (1927). Dermen (1933) observed vacuolation of the nucleoli as one of the most important features in living as well as in fixed material of *Callisia*, *Paeconia*, and *Pinus*. He thinks that the vacuoles are not permanent features of the nucleoli; vacuolation may be considered a normal phenomenon and vacuoles may appear and disappear normally.

From the existing data and the present material the writer agrees with Dornen that vacuolation is a normal feature of the nucleoli in plants, and their absence in some of the nucleoli of the resting stages in wheat suggests that the flow of nucleolar material from the nucleoli has not yet started. This may also go to explain the observation of Fikry (1930) that vacuolation of the nucleoli becomes more apparent in the prophase stage of *Rumex scutatus*, rather than his explanation that it is due to treatment.

In addition to the vacuoles, one or more shining crystalloid bodies were present in the nucleoli. They were not always seen in the resting or the early stages, but their presence was particularly noticeable in the late prophase stages, where in some cases they could be counted as fifteen or more. Such crystalline inclusions of the nucleoli have also attracted the attention of several workers before. Cleland (1922) observed them in the nucleoli of *Oenothera franciscana*, and de Mol (1926b) in the nucleoli of the root-tip cells of *Hyacinthus*. Sheffield (1927) reports the presence of a single large highly refractive crystal-like structure within a large vacuole as a constant feature of the nucleolus of the premeiotic resting nucleus of *Oenothera rubricalyx*, whereas the nucleolus of the early prophase contains as many as a dozen small crystalloids, each lying within its own vacuole.

Latter (1926) holds the nucleolar body contained within the nucleolus as an area where the mixing and elaboration of nucleolar and cytoplasmic material takes place. Gates and Latter (1927) are of opinion that the occurrence of crystal bodies in the nucleoli of the resting pollen mother-cell nuclei of *Lathraea* suggests the possibility of later formed nucleolar bodies being derivatives of the crystalline inclusions. It is very difficult to say anything about the chemical nature of these crystalline inclusions, but the fact that in the present material the increase in their number is more or less concomitant with the gradual disappearance of the nucleolus suggests that these crystalline inclusions are of the nature of reserve material in the nucleolus, being the last to be utilized by the cytoplasm or the chromosomes.

There was no clear area seen around the nucleolus in wheat, contrary to the observation of Latter (1926) on *Lathyrus odoratus*, and the writer agrees with McClung (1929), Dornen (1933), and Mazundar and Datta (1935) that this area is an artifact. This has also been shown by Fikry (1930), who observed a distinct relationship between good fixation and smallness of this zone.

HETEROTYPIC PROPHASE

The first indication of the approaching heterotypic prophase is shown by slight increase in the size of the nucleus. With this increase in size the granular appearance of the nucleus undergoes a readjustment in its 'granules', situated in linear series, resulting in the appearance of definite chromatin threads. The nucleus can now be described in its early leptotene stage. At this stage also, like the resting stage, from one to four nucleoli (Pl. XIII, Fig. 1) have been seen in tetraploid wheat, and the maximum number observed in the hexaploid

was also four. Dermen (1933) in *Callisia* and *Pinus*, and Sheffield (1927) in *Oenothera*, have observed the presence of more than one nucleolus at this stage. Fusion of the nucleoli at this stage is very common, as seen in Pl. XIII, Figs. 2 and 3, and Pl. XV, Fig. 49, shows the fusion of two big nucleoli in *T. vulgare*, and (Pl. XIII, Fig. 3) in *T. dicoccum*. From Pl. XIII, Fig. 2, it is clear that the four nucleoli of *T. dicoccum* first fuse into two separate pairs, and then these pairs fuse into one big nucleolus which can be termed a compound nucleolus.

Contrary to the observations of Latter (1926) in *Lathyrus odoratus*, Sheffield (1927) in *Oenothera*, Fisk (1927) in *Zea mays*, Gates and Latter (1927) in *Lathraea*, Latter (1932) in *Malva sylvestris*, Mazumdar and Datta (1935) in *Hibiscus mutabilis*, and Nandi (1937) in rice, no budding or fragmentation of the nucleolus was seen in the present material. The writer has observed the process of fusion of nucleoli only, as described by Fikry (1930) in *Rumex scutatus*, Sorokin (1929) in Ranunculaceous plants, and Kaufmann (1934) in *Drosophila melanogaster*, where he is of opinion that the single nucleolus visible in some cells results from the fusion of two original nucleoli, one associated with each of the two sex chromosomes. Moreover, Dermen (1933) observed the process of fusion only between two nucleoli at prophase in living tissue of *Callisia*, but never observed fragmentation. Besides this, as already expressed by Dermen (1933) and as also observed by the writer in the present material, the abundance of higher numbers of nucleoli in early stages of nuclear development and lower numbers in later stages must mean one thing only, that this decrease of number and increase in volume of nucleoli comes about through fusion. Thus from these facts it is concluded that the process of fusion of the nucleoli is a natural phenomenon. Budding of nucleoli is only due to treatment and is considered therefore an artifact, as already expressed by Nandi (1937) in rice.

Tables I and II give the measurements of the relative volumes of nucleoli in the leptotene stage of the hexaploid and tetraploid wheats. The measurements are taken from figures drawn by camera lucida magnified 2,900 times.

From these measurements it is obvious that the mean of the total nucleolar volume is greatest where there is a single compound nucleolus, and diminishes progressively where two, three, or four separate nucleoli are present in the nucleus. These observations are in perfect harmony with those of de Mol (1926 *b*) in a diploid variety of *Hyacinthus* where he says 'that the surface and the volume of the two simple nucleoli are equal to those of the complex nucleolus. The calculations, however, indicate clearly that both values of the complex nucleolus are much greater. As repeatedly in nuclei, lying beside each other, the size of the complex and simple nucleoli occurs in a similar ratio, it may be supposed perhaps that a part of the material is used up or is lost during fragmentation. The same may be said about tri- and tetra-nucleolar varieties.'

The reason given by de Mol for the increase in volume of the complex

nucleolus over the total volume of the nucleoli from which it is composed is that perhaps a part of the material is used up or is lost during fragmentation, but on the contrary the writer attributes the increase in the volume of the complex nucleolus to growth, leading directly to corresponding increase in

TABLE I

Nucleolar Volume in the Leptotene Stage of a Hexaploid Wheat*

No. of nucleoli per cell.	No. of cells.	Maximum volume in c.mm.	Minimum volume in c.mm.	Mean in c.mm.
Single nucleolus	9	1437.3	623.8	1021.7
Two nucleoli	11	1178.5	628.7	885.5
Three nucleoli	7	1039.8	560.4	709.8
Four nucleoli	1	737.0		737.0

TABLE II

Nucleolar Volume in the Leptotene Stage of Khapli Emmer, a Tetraploid Wheat*

No. of nucleoli per cell.	No. of cells.	Maximum volume in c.mm.	Minimum volume in c.mm.	Mean in c.mm.
Single nucleolus	12	905.1	523.8	704.0
Two nucleoli	13	730.6	335.9	555.1
Three nucleoli	13	705.6	216.1	486.9
Four nucleoli	6	603.3	309.8	473.8

(* Calculated from measurements of diameters of spherical nucleoli in camera lucida drawings magnified 2,900 times.)

diameter of the complex nucleolus after it has been formed by fusion of the other nucleoli. Such an increase in the volume of the complex nucleolus has been observed by Selim (1930), when he supposed that 'it is very probable that the division of the nucleolus is due to the relative increase of the nucleolar material within the mother nucleolus'.

At leptotene, some of the delicate threads are always attached to the nucleolus. Pl. XIII, Fig. 1, shows the early leptotene stage of the tetraploid wheat with four nucleoli. Each nucleolus is attached to a single thread at its terminus. In rice, Nandi (1937) observed that whenever there were two nucleoli, the two bodies with their chromatic threads remained situated near the point of attachment between the two nucleoli. Pl. XIII, Fig. 3, is another stage where the attachment of four threads at their terminus to the nucleolus, which is being formed by the fusion of two nucleoli in the tetraploid wheat, is seen. In the hexaploid wheat, Pl. XIII, Fig. 21, shows a single large compound nucleolus with six leptotene threads attached to it at their terminal satellite, which are also clearly seen in the photomicrograph (Pl. XV, Fig. 50). These attachments are quite in accord with the four satellite chromosomes in the tetraploid and six in the hexaploid, observed in the somatic metaphases.

The association of the nucleolus with the chromosomes has been observed

by several workers. Farmer, as early as 1895, observed in the spore mother-cells of various Hepaticae that the nucleolus was associated with the chromosomes in an 'unmistakable and remarkable manner'. Wager (1904), working on the root-tip cells of *Phaseolus*, figures the nucleolus connected to the nuclear network by means of very fine threads.

Latter (1926), after a prolonged detailed examination of the pollen mother-cells of *Lathyrus odoratus*, found one loop of the spireme definitely in contact with the persistent deep-staining portion of the nucleolus which she calls the nucleolar body, and which was not observed later than the brochonema stage. A similar relation was shown in *Oenothera* by Sheffield (1927), and Gates and Sheffield (1929). Gates and Latter (1927) showed it in *Lathraea* and Latter (1932) in *Malva sylvestris*. Selim (1930) observed it in rice, and Percival (1932) showed it in the hybrid *T. monococcum* × *T. aegilopoides*.

Nandi (1937) observed in rice two of the leptotene threads always attached to the nucleolus by their terminal knobs, which presumably according to him represent the nucleolar body first described by Latter (1926) in *Lathyrus*, and the nucleolar organizing body in the satellite-chromosome of *Zea* by McClintock (1934). In a more recent paper Nandi (1936) describes two pairs of chromosomes with terminal satellites attached to the nucleolus from the early prophase stages of meiosis until the time of disappearance of the nucleolus at prometaphase.

Satellites or trabants were first discovered by S. Navashin (1912) on the chromosomes of *Galtonia*, where he says that they are at first situated on the surface of the nucleolus, but later become attached to fine threads sent out from the body of certain chromosomes. Dermen (1933) observed that in *Callisia* the satellite-chromosome pair holds the nucleolus at its satellite end, and that the satellites are normal components of some chromosomes, and not free bodies picked up by chromosomes from the surface of the nucleolus. According to Smith (1933), the satellites in *Galtonia candicans* are found paired and both in contact with the nucleolus in the prophases of heterotypic division.

Kaufmann (1934) reports the Y-chromosome of *Drosophila melanogaster* connected with the nucleolus into a short swollen, sometimes almost spherical body, and a long rod-like portion which comprises the remainder of the chromosome. Often a delicate chromatic thread traverses the substance of the nucleolus connecting the two parts, which in the case of the X-chromosome may be removed from each other by a distance equal to the diameter of the nucleolus. A similar connecting thread is often seen in the X-chromosome, bridging the proximal secondary constriction after the disappearance of the nucleolus. Nemec (1929), in centrifuging the root-tips of *Zea*, has shown it to be highly probable that the nucleolus is connected with the reticular threads. Recently Frolova (1936) has described in the salivary gland nuclei of *Drosophila* a definite body, the chromocentre in the nucleus, to which all the chromosomes are attached by their proximal ends on one hand, and on the other the chromocentre is also connected to the nucleolus by a delicate thread.

There are several others who have shown a similar connexion but no attempt will be made to enumerate them here, as the essential points of most of these workers have been embodied in a paper by Gates (1937), but the writer cannot help emphasizing the remarks of Heitz (1931*b*), who is of opinion that all plants probably have satellited chromosomes coinciding with the number of genomes present in a particular species and that the satellite-chromosomes give rise to the nucleoli in telophase.

The leptotene threads now start to conjugate in pairs. As shown before, the attachment of the six leptotene threads at six separate points to the compound nucleolus in the hexaploid, and of four such threads at four separate points in the tetraploid wheat, or of four separate leptotene threads to four separate simple nucleoli in the tetraploid wheat, suggests very strongly that before the pairing of the homologous leptotene threads, they are situated apart from each other, and by some process or other which involves a series of movements not understood at the present moment, these threads are brought gradually closer and closer, until they show pairing and enter into the zygonema stage. Whatever the series of movements or the processes involving the union of homologous threads may be, there seems one thing clear, that along with the pairing of homologous threads there is going on the fusion or, in other words, the pairing of nucleoli, and judging from the fact that there are two large and two small nucleoli seen as the maximum in the root-tip and pollen mother-cells of a tetraploid wheat, and correspondingly four large and two small in the hexaploid wheat, each thus representing a single genom to which it belongs, it is suggested that such a pairing of the nucleoli appears most probably to be a pairing of homologous nucleoli. In addition to this, the process of pairing of such nucleoli throws light on the question of the different kinds of nucleoli observed by various investigators. For instance, Wilson (1925) has provisionally classified the nucleoli into plasmosomes or true nucleoli, and karyosomes or chromatin nucleoli, but until very recently there has been no clear evidence of two types of nucleoli in the cells of higher plants, according to Gates (1932).

According to Yamaha and Sinotô (1925), the nucleolus in general can easily be distinguished microchemically from the chromosome. Zirkle (1931) from his studies concluded that the two variably distinct substances which have often been reported as constituting the nucleolar apparatus of living cells are especially distinct in *Pinus*. Neither substance has fixation or staining characteristic of chromatin. Fikry (1930) says if staining capacity or colour reaction could be used as indications of loss or otherwise of chromatin from the nucleolus up to the diffuse stage in the pollen mother-cells of *Rumex scutatus*, then it is evident that the loss is either very small or nil.

Hedayetullah (1933), in crossing some of the varieties of rice, found that the pollen of a variety with two large nucleoli when crossed with the variety with one large nucleolus or with the variety with two unequal nucleoli gave a hybrid with two unequal nucleoli in the pollen mother-cells. Dearing

(1934) holds that the intensely and homogeneously stained nucleolus in *Amblystoma tigrinum* is composed of peripheral chromatin granules and a central reticulum; that the nucleolus exhibits an achromatic and a chromatic phase; the former manifests itself in late prophase, metaphase, anaphase, and early telophase, while the latter occurs in middle and late telophase, interkinesis, and early prophase.

In the present work, although no distinct differentiation in the staining capacity has been observed, on de-staining the nucleoli have always been found constantly to lose their stain more rapidly than the chromosomes, appearing very pale in colour; thus supporting the view that the deeply staining cover of the nucleolus is not of chromatin nature, as already expressed by Zirkle and Yamaha¹ and Sinotô, and that it is quite distinct from the yellow ground substance in its composition as held by Gates (1907).

The nucleoli in the pollen mother-cells of wheat are related to separate genomes and may differ from each other in their internal constitution; this may account for the differences in their staining capacity or the varying quantities of plastin and chromatin observed by other workers. Photomicrograph (Pl. XV, Fig. 46) shows the amphitene condition, when both paired and unpaired threads can be seen. At *a* and *b* are two leptotene threads connected by their terminal satellites to the nucleolus, and at *c* are seen two other threads which are lying parallel to one another, with their satellited knobs more or less joined together. The threads *a* and *b* are homologous threads which have not yet paired, while the two threads lying parallel to one another at *c* have more or less paired at the satellited end, while for their remaining part they are lying parallel to one another. Hence it is concluded that the pairing in wheat starts evidently at the ends of the threads, and gradually proceeds farther to the remaining parts of the homologous threads, which have by this time come to lie parallel to each other.

In rice Nandi (1937) finds that the unpaired leptotene threads with their free ends begin to run parallel to one another, and then they pair closely with each other, here and there; the mode of pairing is parasynaptic and commences at the ends of the chromosomes. A similar type of pairing has been described by Huskins and Hearne (1931) in oats, by Koshy (1934) in *Allium*, and Naithani (1937*b*) in *Hyacinthus*; and from the present material the writer agrees with Catcheside (1931) that synapsis commences at the ends of the chromosomes and proceeds towards the attachment constriction.

The further pairing of the threads seems to be a very gradual process, and it is accompanied by a gradual shortening and thickening of the individual threads. It may be mentioned here that the tight synizetic knot described by various writers has never been noticed in the present material and the writer thus agrees with Fikry (1930) that the presence of such a knot is not a natural phenomenon, but is due to the action of the treatment of the nucleus, which at this stage is extremely sensitive to the action of reagents.

The pairing is complete at the zygonema stage, and the double threads at

this stage are present in the haploid number. Consequently only two bivalents (Pl. XIII, Fig. 4) in the tetraploid and three (Pl. XIII, Fig. 22, and Pl. XIV, Fig. 43) in the hexaploid parent and hybrid are seen attached by their terminal satellites to the nucleolus, which is vacuolated and granulated. Pl. XV, Figs. 45 and 51 are photomicrographs of tetraploid and hexaploid wheats respectively, showing the attachment of two bivalents in the former and three (marked by arrow) in the latter by their terminal knobs at this stage.

The beaded appearance due to the presence of chromomeres, described by so many workers, was not at all noticed, but on the contrary the spiral nature of the bivalent threads at this stage was very clear, as shown in photomicrograph (Pl. XV, Fig. 48, marked by arrow). It is just possible, as already suggested by Gates and Nandi (1935) in *Oenothera*, and Naithani (1937b) in *Hyacinthus*, that the nodes and internodes formed by the twisting about each other of the two spiral threads may, owing to optical illusion, give the false appearance of chromomeres. The gradual shortening and thickening of the threads finally leads to the pachytene stage (Pl. XIII, Fig. 23). The pairing is now complete. The loop formation of the bivalents, described by Cleland (1922) in *Oenothera franciscana*, by Latter (1926) in *Lathyrus odoratus* and by Gates and Nandi (1935) in *Oenothera* at this stage, were not at all observed in the present material.

In the later stages the behaviour of one bivalent attached to the nucleolus is different from the behaviour of the other similarly attached, both in tetraploid and hexaploid wheats. Pl. XIII, Fig. 5, and Pl. XIII, Fig. 24, show the diplonema stages in tetraploid and hexaploid wheat respectively. In Pl. XIII, Fig. 5, there is shown one bivalent attached by its terminal satellite to the nucleolus, while the other bivalent is lying close to a deeply stained body attached to one edge of the nucleolus. Similarly in the next diagram and photomicrograph (Pl. XV, Fig. 47, pointed by an arrow), one bivalent and the deeply stained body are shown attached to the nucleolus. When it was seen for the first time, it was rather difficult to give an interpretation of this deeply stained body which was constantly found in many preparations. But later it was observed that the chromosome is normally attached by a very fine thread to the satellite, which remains attached to the nucleolus as shown in Pl. XIII, Fig. 7, which is the early diakinesis stage. As this thread is very long and delicate it naturally breaks in some of the preparations, and thus in tetraploid wheat a single bivalent and another body, really the separated satellite, are seen attached to the nucleolus in many of the preparations.

Similarly in the hexaploid wheat, Pl. XIV, Fig. 26, shows the two bivalents attached to the nucleolus by their deeply stained terminal satellites, while the third bivalent is stretched into a thread which is carrying the deeply stained satellite at its tip attached to the nucleolus. The same is clear from the photomicrograph (Pl. XV, Fig. 52, pointed by an arrow). Pl. XIII, Fig. 25, shows similarly the two bivalents and the deeply stained body (satellite) attached to the nucleolus where evidently the thread has been broken. Pl. XIV,

Fig. 37, is diakinesis stage of the hexaploid hybrid showing the attachment of three bivalents to the nucleolus. Here also the delicate thread connecting the satellite with the body of the bivalent has been broken (indicated by an arrow). Thus it is seen that one of the bivalents both in tetraploid and hexaploid wheat is stretched into a fine thread carrying at its tip the satellite which remains attached to the nucleolus.

Kaufmann (1934) observed the Y-chromosome of *Drosophila melanogaster* which is connected with the nucleolus and is divided by it into a short, swollen, sometimes almost spherical body, and a long rod-like portion which comprises the remainder of the chromosome. Often a delicate chromatic thread traverses the nucleolus connecting the two parts which, as in the case of the X-chromosome, may be removed from each other by a distance equal to the diameter of the nucleolus. Baranov (1926) says that the satellites of *Drimopsis maculata* were attached to the nucleolus in the prophase, and in later stages were picked off by their respective chromosomes. In this case, as already suggested by Gates, evidently the delicate thread connecting the prophase chromosome to its satellite was broken in the process of fixation. Dearing (1934) describes a similar thread connecting the satellites to the chromosome, in the case of *Amblystoma*.

The nucleolus in wheat diminishes gradually in size from zygotene onwards, until it totally disappears at late diakinesis. This is in contradiction to the observations of Dermen (1933) in *Callisia* and *Pinus* where no diminution of the nucleolus was observed from the resting stage, when it reaches its maximum value, until the end of prophase before the nuclear membrane disappears. It may also be pointed out here that in the present material the nucleolus reaches its maximum size in the leptotene stage. Ordinarily the number of nucleoli from zygotene onward is always one, but in one preparation of the tetraploid wheat two nucleoli were seen. The nucleoli here evidently have failed to fuse from the very early stage, and a similar observation in rice is also described by Nandi (1937).

The nucleoli at this stage are very much granulated and vacuolated also; they keep their spherical shape and take any position inside the nucleus and the appearance of these crystallized bodies becomes more and more prominent the nearer the nucleolus approaches the limit of disappearance. When two nucleoli were observed at diakinesis, their disappearance was more or less simultaneous, and like rice (Nandi, 1937) no budding, fragmentation, or parting of granules into the cytoplasm was observed at the time when the nucleolus disappears.

Contrary to the observations of Nandi (1937) in the Indian rice, Basantra Bahar, no univalents were observed at diakinesis in the present material. The individual chromosomes continue to contract in size throughout diakinesis. The bivalents are scattered peripherally in the nucleus and not grouped around the nucleolus as described by Sax (1922) in wheat. With extreme contraction the chromosomes finally become smooth in surface and their

stainability is increased, while that of the nucleolus is decreased, suggesting that the stainable material of the nucleolus has passed to the chromosomes.

Pl. XIII, Fig. 9, is a prometaphase of the tetraploid wheat. The nuclear membrane and the nucleolus have disappeared, but comparing the size of the bivalents at this stage with those of the metaphase (Pl. XIII, Fig. 10) it is obvious that they have not undergone complete condensation and contraction. Their ring- or rod-shaped structures are quite evident now. Although they have not undergone a complete contraction, it is not possible to distinguish the satellited chromosomes, the satellites of which appear to have become merged into the body of the chromosome, leaving no trace of the fine thread which was connecting the satellite with the body of one of the bivalents. As the intermediary stages have not been seen, it is concluded that the change which the bivalents undergo from diakinesis to prometaphase is a very rapid one. At metaphase the condensation of the bivalents which are situated on the equator of the spindle is complete. Fourteen bivalents have always been counted in the tetraploid (Pl. XIII, Fig. 10) and 21 in *T. vulgare* (Pl. XIV, Fig. 27) and the hexaploid hybrids (Pl. XIV, Fig. 38).

Although the bivalents show considerable differences in their size, it was not possible to classify them into different size groups, due to these differences merging one into the other. Contrary to the observation of Cleland (1922) in *Oenothera franciscana*, where he describes a prominent black staining little body, the endo-nucleolus, which outlasts the nucleolus in metaphase, no such body was observed in wheat, and although a vigorous search was made to distinguish the satellite chromosomes from the rest, it was not possible to do so at this stage.

The polar view of the late anaphase (Pl. XIII, Fig. 11) shows that the homologous chromosomes are equally distributed on both sides of the equatorial plane, each chromosome on one side having a recognizable homologue on the other side. Thus the distribution of the chromosomes to two poles is always normal, and the two nuclei in the telophase are normally constituted. Interkinesis is marked by the appearance of the cell wall, and by the rapid growth and enlargement of the two daughter nuclei. The spindle fibres gradually disappear between the two nuclei and the individual chromosomes become more elongated and assume a more or less parietal position, being connected to one another by means of anastomosing strands. Contrary to the observations of Latter (1926) in *Lathyrus odoratus*, where no nucleoli are formed between the two nuclear divisions, definite nucleoli arise in wheat, three being the maximum number in hexaploid and two in tetraploid wheat (Pl. XIV, Fig. 28 and Pl. XIII, Fig. 14) respectively. These nucleoli arise as definite bodies in contact with the chromosomes, although no definite connexion could be established with any particular chromosome at this stage.

Van Camp (1924) is of opinion that the nucleoli originate from the chromosomes at telophase in the form of small globules which later by fusion form one large nucleolus. Gates and Sheffield (1929) observed in *Oenothera*

rubricalyx that small spherical faintly staining nucleoli appear to arise *de nova* in contact with the chromosomes. Several may appear in each daughter nucleus. Heitz (1931b) attributes the origin of the nucleoli to the satellite chromosomes, especially on and around the achromatic thread that connects either a satellite or a constricted arm with the chromosome. McClintock (1934) thinks that the nucleolus is organized in the telophase by an enlarged morphologically distinct deep staining chromosomal body which appears at a definite position on one chromosome of each haploid complement of *Zea mays*. Nandi (1937) is of opinion that the nucleolus-like globules at the telophase represent the original nucleolar material of the mother nucleus, which passed into the metaphase chromosomes and was carried as such to the daughter nuclei in the anaphase chromosomes. In the heterotypic telophase the nucleolar material present in each chromosome gets liberated as small globules which, under the influence of the nucleolar body present in one chromosome of the haploid complement, is organized into a definite nucleolus that remains associated with one particular chromosome.

HOMOTYPIC DIVISION

Before the formation of homotypic spindles the condensing chromosomes take up more definite form, losing the appearance of anastomosing threads. The nuclear membrane and the nucleoli gradually disappear. The chromosomes take a deeper stain due to their matrices becoming more conspicuous about their chromonemata and they take their position now on the equatorial plate of the spindle (Pl. XIII, Fig. 12, Pl. XIV, Figs. 29 and 36). At this stage they appear long, slender, curved and more or less bi-armed threads, resembling more the somatic chromosomes than those of the first meiotic division. Their number at this stage is fourteen for tetraploid and twenty-one for hexaploid wheats. The satellited chromosomes were not recognizable.

In the anaphase the two chromatids move normally to the opposite poles. Pl. XIII, Fig. 16, is a late anaphase of the tetraploid wheat. In the hexaploid hybrid (Pl. XIV, Fig. 44) some lagging chromosomes are observed, but the formation of normal tetrads in the latter stage shows that the laggards ultimately reach the poles. Similar laggards were observed by Latter (1926) in *Lathyrus odoratus*. The grand-daughter nuclei are formed in the same way as the daughter nuclei. They grow rapidly and the individuality of the chromosomes is soon lost, giving the appearance of a delicate reticulum (Pl. XIV, Fig. 35) interlaced with particles of chromatin of various sizes.

Nucleoli are developed in the grand-daughter nuclei, with the development of anastomosing strands between the chromosomes in the same way as in the heterotypic telophase. Cleland (1922) observed one to several nucleoli in the daughter nuclei of *Oenothera* at this stage. In wheat one or two nucleoli in the tetraploid and one, two, or three in the hexaploid have been observed at this stage. Pl. XIV, Fig. 35, shows all the four cells in a tetrad of tetraploid wheat with two nucleoli. Pl. XIII, Figs. 13, 15, and 17, are the different

stages of the quartets showing two nucleoli in the tetraploid wheat. Pl. XIV, Figs. 30 and 33, are tetrad cells from the hexaploid wheat showing three nucleoli in each. Pl. XIV, Fig. 30, shows the fusion of two nucleoli from a very early stage, while the third nucleolus is separate. It shows that the fusion of the nucleoli may begin in early telophase.

POLLEN GRAINS

Pl. XIV, Figs. 39, 40, and 41 show the young pollen grains of the hexaploid hybrid. They are smooth and more or less oval and somewhat irregular in form and size. They are characterized by a single germ pore which is closed by a minute lid or operculum, which is pushed aside at the time of development of the pollen tube. This germ pore arises as a small bulge in the form of a beak (Pl. XIV, Fig. 39). Later this beak is surrounded by a thickened area which forms the thick rim of the germ pore (Pl. XIV, Figs. 40 and 41). The occasional formation of two germ pores, as described by Percival (1926) in the pentaploid hybrid, *A. ovata* × *T. vulgare*, was not met with at all.

CYTOMYXIS

Pl. XIV, Fig. 34, shows the phenomenon of cytomyxis at the zygotene stage of the hexaploid hybrid. A part of the chromatin material is being injected into the adjacent cell. The nuclear membrane of the cells showing cytomyxis is very delicate, and the cytoplasm also shows a little shrinkage. Cytomyxis was described by Gates (1911) in *Oenothera gigas*, when he applied this term to the process of extrusion of chromatin from the nucleus of one pollen mother-cell into the cytoplasm of an adjacent cell. Gates and Latter (1927) observed that in addition to the occurrence of cytomyxis in the prophase of *Lathraea*, it also occurs during interkinesis. Kattermann (1933) described it in the pollen mother-cells of *Triticum* × *Secale* hybrids, Kihara and Lilienfeld (1934) in hybrids between *Triticum* and *Aegilops*, and Percival (1930) in the hybrid *A. ovata* × *T. monococcum*. Cytomyxis is a very familiar phenomenon in many plants, particularly in the grasses.

Church (1929) has described the various types of grasses displaying different kinds of cytomyxis. The first type are those where the phenomenon is seen only in the spireme stage, but with the resultant extrusions persisting in later stages. These are *Andropogon scoparius*, *Digitaria sanguinalis*, *Spartina michauxiana*, and *Festuca rubra*. The second type exhibits such chromatin loss to the extent that the process is still manifested in diakinesis. Among these he describes *Ammophila breviligulata*, *Alopecurus pratensis*, *Phalaris canariensis*, *Paspalum muhlenbergii*, and *Echinochloa Crus-galli*. The third type is that where it is observed in abundance at diakinesis as in the dichotum type of *Panicum*. Church is of opinion that the greatest amount of cytomyxis is correlated with the greatest amount of irregularities in the maturation divisions, and an excessive amount of cytomyxis in all stages of the prophase may result in irregularities in the heterotypic metaphase in which chromosomes on

or off the spindle may be partly stranded in adjacent cells. In the case of *Miscanthus sinensis* var. *zebrinus*, the heterotypic spindle has been seen stranded between two mother-cells and cytomyxis was observed here in all stages.

From the present study, and also from the fact that cytomyxis so far as wheat is concerned was before noted only in the hybrids *Triticum* × *Secale* and *Triticum* × *Aegilops*, the author agrees with Church in saying that even if the phenomenon can be proved not to be an exclusively hybrid characteristic its occurrence is manifestly associated with hybrids, although the probability of its also being of pathological nature cannot be totally excluded. Pl. XIV, Fig. 42, shows an abnormal stage of the late anaphase from the hexaploid hybrid, where instead of the expected twenty-one chromosomes going to each pole, about forty-two were observed.

THE PROBLEM OF RUST RESISTANCE

The problem of immunity or susceptibility of wheat to the attacks of rust fungi is a very complicated one, which in spite of the efforts of geneticists, mycologists, and physiologists in the last fifty years, is standing unshakable so far as its control is concerned. Geneticists have tried to evolve rust-resistant varieties of wheat which appear to have done well for several years, but later on seem to have lost their rust-resistant quality. Pole-Evans (1911) observed that certain wheats recommended for rust-resistant qualities holding good for several years in succession had fallen prey to rust in course of time.

The general deterioration and reversion of certain varieties of wheat, which farmers allege will, according to Engledow (1927), raise for the students of heredity certain problems; the solution of which will call for special and difficult experiments. In the present work too, the 'Sherbati' hybrids which have for several years held a position of pride amongst the wheats of C.P. and Berar, appear to have lost their rust-resistant capacity in due course. These facts not only add to the complexity of this problem, but open a way for a reasonable question as to the cause for this deterioration.

Marshall Ward (1902) found no resemblance between the infection curve—as it may be termed—and the curves of the sizes or of the numbers of stomata, of the sizes and numbers of hairs, of the area of the leaves, or in short the curves of any other structural factors of the leaf.

In his studies on the inheritance of disease resistance, Biffen (1907, 1912) observed that on crossing immune and susceptible varieties the resulting offspring are susceptible; these on selfing produce immune and susceptible descendants in the proportion of one of the former to three of the latter, the degree of susceptibility being variable. Moreover, when the degree of susceptibility differs in the two parents, the hybrid resembles the more susceptible parent in that respect, and among the descendants of such hybrids the degrees of susceptibility appear in the usual Mendelian ratio of one slightly to three very susceptible individuals. The resulting immune forms breed true to this characteristic in the succeeding generation. Any factor altering in any way

the metabolic processes of the plant in turn alters the degree to which it is attacked by yellow rust and probably other fungi as well, and immunity was found to be independent of any recognizable morphological feature.

Marryat (1907) attributed the immunity to disease to the production of certain toxins and antitoxins by host or parasite, or both, which are mutually destructive. Butler (1905) considered that the inheritance of resistance and susceptibility to rust may hold for a given rust in a particular locality; it may or may not hold when the wheat is exposed to the attacks of a second species of rust or when it is transferred to another locality. His experience in India seems to show that resistance to yellow rust (*Puccinia glumarum*) does not imply resistance to orange rust, *P. triticina*, and it is certain that these characters alter with change of locality. He observed that several hybrids resistant to Australian rusts have proved susceptible to the same rust in India.

Pole-Evans (1911), from his studies on the problem of rust resistance, concluded that the rust which has passed through a hybrid plant produces a far more severe infection than the rust from the susceptible parent, that is, the pathogenic properties of the rust are increased after its sojourn in a favourable host. Whether this is simply due to the fungus being in a more vigorous condition for attack or whether there are more subtle reasons for it he could not say, but he is definite that such hybrid plants may play a very important part in the transmission of rust organisms from susceptible to immune varieties, that is, the hybrid acts as an intermediary in the passage of the rust from a susceptible to an immune parent.

Stakman and Levine (1922) have shown that *Puccinia graminis tritici* (Pers.) Erikss., and Henn in reality consists of many biologic forms, which can be recognized by their action on different varieties of wheat. According to Stakman and Piemeisel (1917), more than one biologic form of *Puccinia graminis* may occur on the same host in nature, sometimes even on the same plant, and the different strains of the same biologic forms sometimes differ in virulence on the same host; but the differences are usually in degree only. Furthermore they are of opinion that the rate of development of a given biologic form depends on the vigour of the rust strain, the kind, and sometimes the age of the host plant, the amount of light, heat, and humidity. Sunlight, high relative humidity, and moderate temperatures up to about 75° F., are favourable to rust development. Probably the hypothesis of 'bridging' species owed its development to the presence of different biotypes of the same fungus.

According to Hayes, Parker, and Kurtzwell (1920), susceptibility is a dominant character in crosses between resistant Durums and susceptible common wheats, and a recessive character in crosses between resistant Em-mers and susceptible common wheats. Nilsson-Ehle (cited by Hayes, Parker, and Kurtzwell) seldom found distinct dominance of susceptibility. The F_1 generation was intermediate and in other cases resemblance to one or other parent was observed. He explained his results on the basis of multiple factors.

Waldron (1921) has suggested that the linkage of Durum head character and rust resistance in the segregates of a cross between Durum and a common wheat might be due to the linkage, and that crossing-over would produce the occasional resistant Vulgare type which is sometimes found.

Hynes (1926) states that from a cross of Marquis $\times$ Jumillo with Marquis $\times$ Kanred it appears that two chromosomes or parts of chromosomes which contained factors for resistance were obtained from the Durum parent, and were combined with chromosomes of the Vulgare group.

Biffen and Engledow (1926) got a ratio of 1 : 3.07 of resistant to susceptible types in a cross between Michigan Bronze, which is excessively susceptible to yellow rust, and American Club, a highly resistant wheat; but they observed also that this was only a partial explanation of the facts, for they could distinguish two groups of susceptible plants, one of true susceptibles resembling Michigan Bronze in the intensity with which they were attacked, and the other of intermediates with a wide range of susceptibility, but on the whole less than that of the former. Waterhouse (1930), from his experiments in Australia on the inheritance of resistance to Forms 43 and 46 of *P. graminis tritici*, concluded that resistance is due to a single dominant factor. On the other hand, the inheritance of resistance to Form 34 was due to one factor (or more) with clear dominance of susceptibility.

Kostoff (1936) remarks that the investigations carried out by Vavilov showed that 14-chromosome wheats (i.e. the Monococcum group) are most resistant to certain fungus diseases, 28-chromosome wheats (Durum group) being less resistant, and finally those with forty-two chromosomes (Vulgare group) being most susceptible. On the basis of this regularity another question arises. Is there any causal dependence between the chromosome number and susceptibility in wheat plants? According to the law of the nucleo-cytoplasmic ratio 28-chromosome species should have larger cells, and 42-chromosome ones much larger than the 14-chromosome species. Direct investigation showed that this was rarely true. The species with the larger chromosome number are more susceptible and they have larger cells. This suggested the idea that the immunity of the species with small chromosome numbers might be rather a mechanical one than physiological, because the fungus hyphae penetrate much more easily through the larger stomata of the Vulgare group, than through the smaller ones of the Monococcum group. This regularity might have a general significance, but it has not an absolute validity. *T. Timopheevi* represents an especially good example in these respects. It has 28 chromosomes, twice as many as Monococcum; nevertheless it is more resistant and to many more fungus diseases than *T. monococcum*. The amphidiploid plant *T. Timococcum* has as many chromosomes as *T. vulgare*, namely forty-two, but it appears to be as resistant as the parental forms, although this is not a final conclusion because it was based on the reaction of a single *T. Timococcum* plant.

In the present work it is seen that the 'Sherbati' hybrid, a cross between

T. dicoccum 'Khapli' and *T. vulgare*, was rust resistant to begin with, but in the course of time it lost its property as a rust-resistant wheat. In this respect it agrees with the observations of Pole-Evans (1911). The 'Sherbati' wheat is a cross between *T. vulgare*, an awnless but susceptible wheat having forty-two chromosomes, and *T. dicoccum*, a fully awned but perfectly immune wheat having twenty-eight chromosomes, and it is a selection in F_3 by Evans. The F_1 from this cross must have contained twenty-one *Vulgare* plus fourteen *Dicoccum* chromosomes. In meiosis the fourteen *Dicoccum* paired with fourteen of the *Vulgare* chromosomes, while the behaviour of the other seven chromosomes was irregular, thus giving rise to segregates varying from twenty-eight chromosomes to forty-two chromosomes. The F_2 or later segregates with forty-two chromosomes would therefore be made up of twenty-eight chromosomes from *Vulgare* plus 14 from *Dicoccum*.

The present generation of Sherbati wheat has forty-two chromosomes, out of which there should be at the maximum fourteen chromosomes from the *Dicoccum* parent, but from its F_2 up to the present generation the following results of the *Vulgare* and *Dicoccum* segregates, on the basis of the shuffling of their chromosomes in meiosis, could be possible:

F_2 .	Chromosomes 42.	<i>Vulgare</i> 28	+	<i>Dicoccum</i> 14.
In the subsequent generations segregates with the following numbers of chromosomes are possible:				
No. 1		21	+	0
2		20	+	1
3		19	+	2
4		18	+	3
5		17	+	4
6		16	+	5
7		15	+	6
8		14	+	7
9		13	+	8
10		12	+	9
11		11	+	10
12		10	+	11
13		9	+	12
14		8	+	13
15		7	+	14

On the assumption that the fourteen chromosomes from the *Dicoccum* all carry genes for rust resistance, the segregate No. 15 will be fully resistant and bearded, and the quality of rust resistance will decrease from No. 15 to No. 1 segregate. No. 1 segregate will therefore be most susceptible, like its *Vulgare* parent. Now a glance at the photograph (Pl. XV, Figs. 53-67) shows clearly that these segregates, which are possible on mathematical grounds, can be identified by the shape of the ears and the length of the awns. Some of them, as for instance Pl. XV, Figs. 55 and 56, approach very closely to the *Vulgare* parent (Pl. XV, Fig. 53), in these characters, and these will be very suscep-

tible while others are intermediate, and in still others (Pl. XV, Figs. 63, 65, 66, and 67) the awns resemble more the *Dicoccum* parent (Pl. XV, Fig. 54).

From this hypothesis it is clear that there is a direct relation between the length of the awn and the quality of rust resistance, which ultimately appears to depend on the number of chromosomes present from the *Dicoccum* parent. In these segregates plants with longer awns show greater resistance, and they therefore probably possess more chromosomes from the *Dicoccum* parent. It may be remarked here that Sax (1923), working with similar hybrids from forty-two chromosome wheat *T. vulgare* with twenty-eight chromosome wheat *T. durum*, observed also that the true awnless segregates were very susceptible while the segregates with very long awns were resistant to rust, and with an increase in awn lengths the power of resistance increased. He also observed that there was a very high degree of correlation between chromosome number and rust resistance, head type, keel shape, and spikelet shape.

Waldron (1921) and Hynes (1926) have concluded that one pair of *Durum* chromosomes is responsible for the resistance. In the present paper it is concluded that the genes for resistance to a given rust are carried by many of the chromosomes of the *Dicoccum* parent in the 'Sherbati' hybrids. The number of these chromosomes will determine whether a given segregate is fully or partially rust resistant, and in their absence the segregate will be entirely susceptible. Further work is necessary to determine whether all chromosomes of *Dicoccum* are equally concerned in genic resistance to rust, or whether some chromosomes contain more potent genes than others.

In the light of this view, the crosses between fully rust resistant and fully susceptible varieties or species will not give a true 1 : 3 ratio of immune to susceptible, but the hybrids will show different grades of immunity and susceptibility according to the shuffling of the chromosomes. The same was observed by Biffen and Engledow (1926) when they say that the ratio of 1 : 3.07 of immune to susceptible which they obtained was only a partial explanation of the facts, for the susceptible group showed a wide range of susceptibility. Moreover, susceptibility should not always be a dominant factor, but its behaviour will depend on the number of chromosomes carrying genes for rust resistance present in the parent crossed, and this was actually observed by Hayes, Parker, and Kurtzwell (1920) when they found that in some of the crosses it was dominant while in others it was recessive, and also by Nilsson-Ehle who seldom found dominance of susceptibility.

The observation of Butler that several hybrids resistant to rusts in Australia proved susceptible to the same rust in India can be easily interpreted on the ground that in these hybrids also shuffling of the chromosomes carrying genes for rust resistance took place in meiosis, resulting in some of the segregates being fully susceptible, and therefore this behaviour of the hybrids need not necessarily be attributed to the change in locality. Moreover, if it be due to the change in locality, then 'Khapli Emmer' should show such a change from place to place; but this is not the case, as it has been reported to be perfectly

immune from all parts of the world. It may also be added that Marshal Ward as early as 1902 expressed the opinion that the resistance to infection of the immune or partially immune species and varieties is not to be referred to observable anatomical or structural peculiarities, but to internal, i.e. intraprotoplasmic properties, beyond the reach of the microscope, and similar in their nature to those which bring about the essential differences between species and varieties themselves.

SUMMARY

1. The cytology of the following wheats from the Department of Agriculture, C.P. and Berar (India), is dealt with:
 - (a) *Triticum dicoccum* var. *Indicum*, popularly known as 'Khapli C.P.'
 - (b) *T. vulgare* var. *albidum* A1. departmental wheat Ao88, and popularly known as 'Mudya'.
 - (c) A112, A113, and A115, the three sister strains of the hybrid between (a) and (b). Popularly they are known as 'Sherbati hybrids'.
2. The morphology of the metaphysic chromosomes in somatic mitosis has been studied. *T. dicoccum* has two pairs of satellited chromosomes, one of the pairs being tandem satellited. Of the remaining twelve pairs, eleven have one constriction each, whereas the twelfth has two constrictions. The chromosome set of *T. dicoccum* is made up of two different sets, A and B, based on the morphology of their chromosomes.
3. *T. vulgare* has three pairs of satellited chromosomes, one of the pairs being tandem satellited. Of the rest there are 12 pairs with only one constriction, whereas three pairs have two constrictions, and the remaining three pairs have three constrictions each. From the morphology of chromosomes in *T. vulgare* it is evident that no two pairs of chromosomes are identical, and that the forty-two chromosomes represent three different sets, A, B, and C. Further investigation of the chromosome morphology of wheat and allied genera will lead to a better understanding of the origin of *T. vulgare*.
4. From the morphology of the chromosomes of 'Sherbati hybrids' which are hexaploid and are about in their twenty-third generation, it is concluded that shuffling of the parental chromosomes has taken place. They also show six satellited chromosomes, one of the pair being tandem satellited.
5. From the measurements of the single and compound nucleoli in the leptotene stage, it is concluded that the mean total nucleolar volume is greatest when there is a single (compound) nucleolus, and diminishes progressively when two, three, or four separate nucleoli are present. The differences in size and in staining capacity between the nucleoli of one cell may result from their belonging to separate genomes.
6. Four leptotene threads in tetraploid and six in hexaploid wheats are attached at separate points to the compound nucleolus formed by the fusion of smaller nucleoli. In tetraploid wheat the four separate leptotene threads were also observed attached separately to each of the four nucleoli.

7. From diplotene onwards to diakinesis, one of the bivalents attached to the nucleolus both in tetraploid and hexaploid wheats is normally connected by a long and very fine thread to the satellite which remains attached to the nucleolus. At metaphase the satellites become merged with the body of the chromosomes. No chromomeres were seen in the chromosomes of wheats, which are always spirally coiled in structure.

8. The maximum number of nucleoli in the nucleus of *T. vulgare* is six, four big and two small, and in *T. dicoccum* four, two big and two small, both in root-tip cells and pollen mother-cells. The nucleoli are granulated and vacuolated in all stages, and vacuolation is considered to be a normal feature of the nucleoli of wheats.

9. Definite nucleoli arise in the interkinesis stage in contact with the chromosomes, three being the maximum number in hexaploid, and two in tetraploid wheats. In the tetrad stage the nuclei of tetraploid wheat contain a maximum of two nucleoli, and of hexaploid a maximum of three.

10. The occurrence of cytomyxis may be associated with the hybridity in wheat, although the probability of its being of pathological nature is not totally excluded.

11. The various degrees of susceptibility to rust fungi in segregates from the 'Sherbati hybrid' wheats indicate that all the chromosomes of the Khapli parent are probably carrying genes for rust resistance.

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EXPLANATION OF PLATES XIII to XV

Illustrating Dr. G. S. Bhatia's paper on 'The Cytology and Genetics of Some Indian Wheats'.

All figures were drawn at the table level with the aid of a camera lucida. A 2 mm. Zeiss apochromatic, 1.3 aperture, and Zeiss ocular $\times 20$ were employed for all drawings. Magnification of all the diagrams is $\times 2900$ with the exception of Figs. 8, 14, 30, 31, 32, 39, 40, and 41, the magnification of which is 1500.

PLATE XIII

(Figs. 1-17 are of *T. dicoccum*, 'Khapli Emmer').

Fig. 1. Leptotene stage, showing the attachment of four separate leptotene threads to four separate nucleoli.

Fig. 2. Fusion of four nucleoli in two separate pairs at leptotene.

Fig. 3. The attachment of four leptotene threads, at four separate points to the compound nucleolus formed by the fusion of smaller nucleoli.

Fig. 4. Zygotene stage, the attachment of two bivalents by their terminal satellites to the compound nucleolus.

Fig. 5. Diplotene stage, the attachment of two bivalents to the nucleolus. In one of the bivalents the delicate connecting thread is broken off.

Fig. 6. Diplotene stage, the attachment of one bivalent and the detached satellite to the nucleolus.

Fig. 7. Early diakinesis, the attachment of two bivalents to the nucleolus. The delicate thread connecting the satellite with the body of one of the bivalents is also shown.

Fig. 8. Resting stage of the pollen mother nucleus showing two big and two small nucleoli.

Fig. 9. Prometaphase showing fourteen bivalents.

Fig. 10. Metaphase showing fourteen bivalents.

Fig. 11. Polar view of late anaphase I.

Fig. 12. Metaphase II.

Fig. 13. Very young pollen grain with two nucleoli.

Fig. 14. Interkinesis with two nucleoli.

Fig. 15. Different stages of quartets with two nucleoli.

Fig. 16. Late anaphase II.

Fig. 17. As for Fig. 15.

(Figs. 18-25 are of *T. vulgare*.)

Fig. 18. Resting pollen mother-cell showing six nucleoli fusing.

Fig. 19. Leptotene stage showing four separate nucleoli.

Fig. 20. The fusion of two big nucleoli into one compound nucleolus.

Fig. 21. Leptotene stage, attachment of six separate leptotene threads at six different points to the compound nucleolus.

Fig. 22. Zygotene stage, attachment of three bivalents by their terminal satellites to the compound nucleolus.

Fig. 23. Pachytene stage showing the same as above.

Fig. 24. Diplotene stage, three bivalents attached by their terminal satellites to the nucleolus. One of the bivalents has a delicate thread connecting the satellite with the body of the bivalent.

Fig. 25. Diplotene stage, showing the attachment of two bivalents and the detached satellite on the nucleolus.

PLATE XIV

Fig. 26. Diakinesis (*T. vulgare*), the attachment of three bivalents by their terminal satellites to the nucleolus. One of the bivalents shows the delicate thread.

Fig. 27. Metaphase I (*T. vulgare*), showing twenty-one bivalents.

Fig. 28. Interkinesis (*T. vulgare*) with three nucleoli.

Fig. 29. Metaphase II (*T. vulgare*).

Fig. 30. Interkinesis showing fusion of two of the three nucleoli in *T. vulgare*.

Figs. 31 and 32. Six nucleoli (four big and two small) and four nucleoli (two big and two small) of *T. vulgare* and *T. dicoccum* respectively in the resting nuclei of the root-tip cells.

Fig. 33. Quartet with three nucleoli in *T. vulgare*.

Fig. 34. Cytomyxis in the zygotene stage of the hybrid *T. vulgare* × *T. dicoccum*.

Fig. 35. Tetrad of *T. dicoccum* showing two nucleoli in each quartet.

Figs. 36-44 are from the hybrid *T. vulgare* × *T. dicoccum*.

Fig. 36. Metaphase II showing twenty-one chromosomes.

Fig. 37. Diakinesis showing the attachment of three bivalents to the nucleolus. The delicate thread of one of the bivalents is broken.

Fig. 38. Metaphase I showing twenty-one bivalents.

Fig. 39. Very young pollen grain, showing the beak from where the germ pore is formed.

Figs. 40 and 41. Young pollen grains showing a single germ pore in front and side view respectively.

Fig. 42. Late anaphase I, showing about forty-two chromosomes instead of the expected twenty-one at this stage.

Fig. 43. Zygotene stage, showing the attachment of the three bivalents by their terminal satellites to the nucleolus.

Fig. 44. Anaphase II showing two laggards.

PLATE XV

Figs. 48-52. Photomicrographs of *T. dicoccum*.

Fig. 45. Late zygotene showing the attachment of two bivalents by their terminal satellites to the nucleolus.

Fig. 46. Amphitene stage. At *c* the two homologous threads are seen pairing, whereas at *a* and *b* unpaired homologous threads are seen.

Fig. 47. The detached satellite, marked by an arrow, of one of the bivalents is shown attached to the nucleolus. The other bivalent is attached to the nucleolus opposite to the detached satellite.

Fig. 48. The spiral structure of the chromosomes of wheat at zygotone is indicated by an arrow.

Figs. 49-52. Photomicrographs of *T. vulgare*.

Fig. 49. Shows the fusion of two big nucleoli into one compound nucleolus.

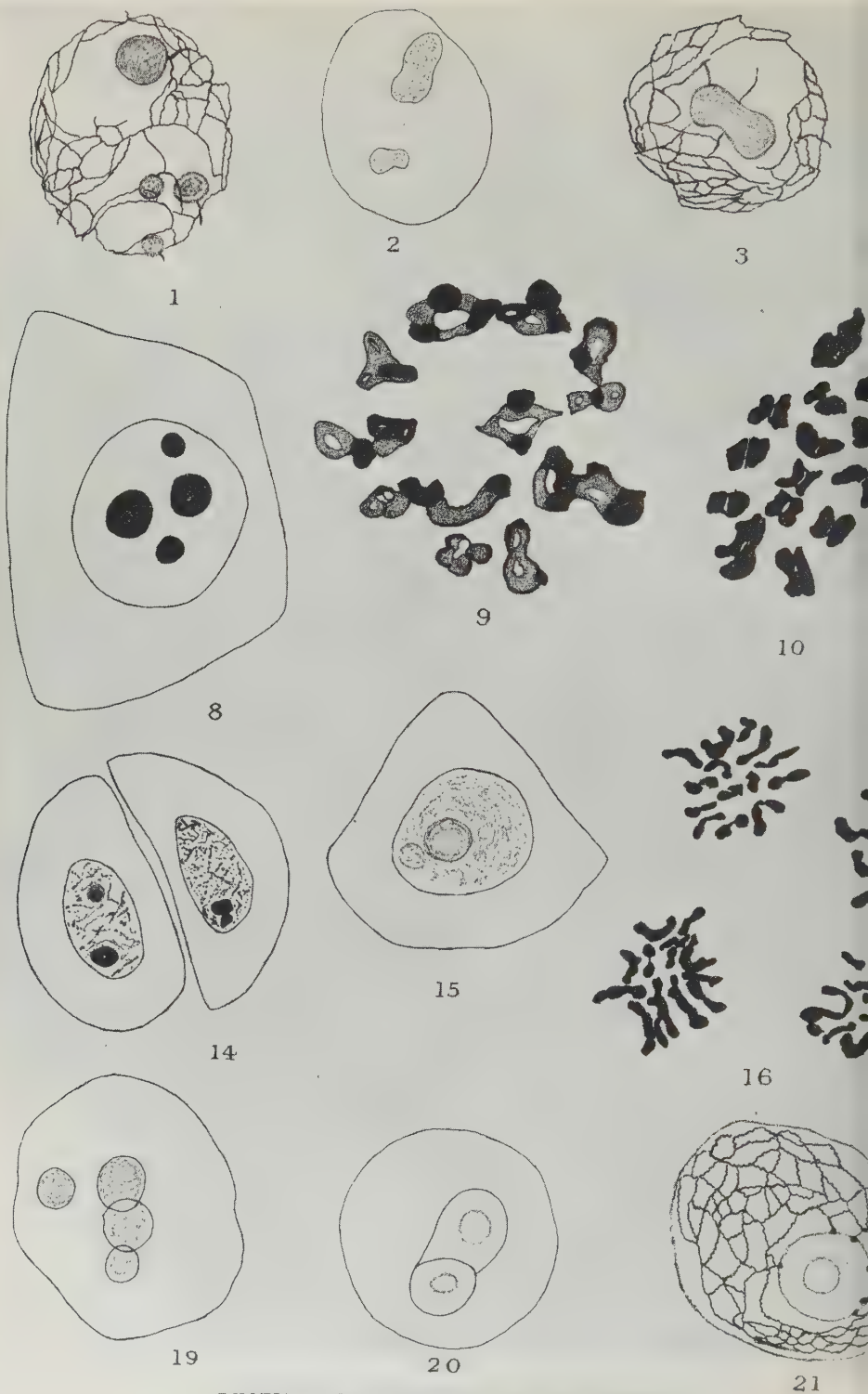
Fig. 50. Shows the attachment of six leptotene threads at six separate points, as indicated by arrows, to the compound nucleolus.

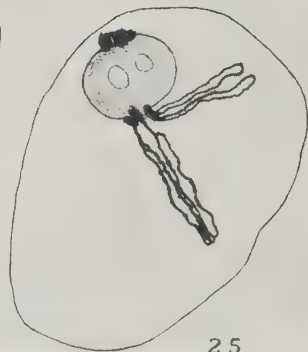
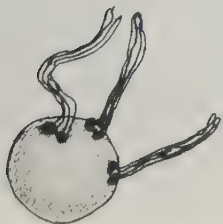
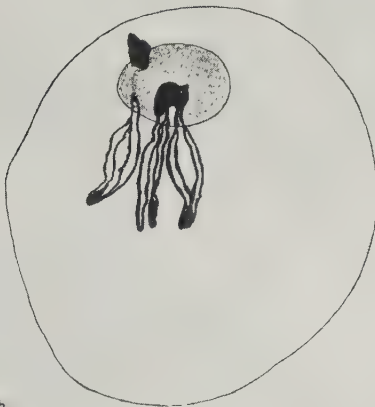
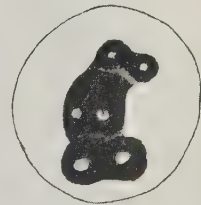
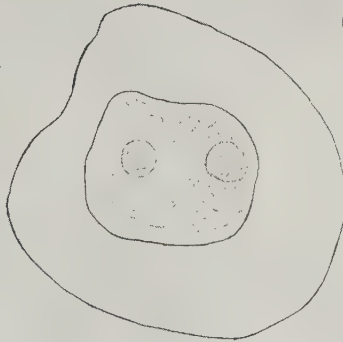
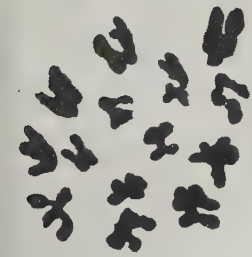
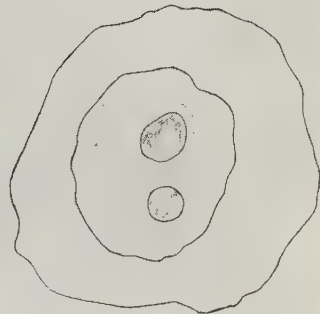
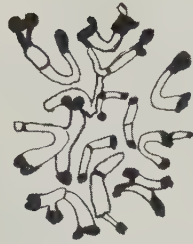
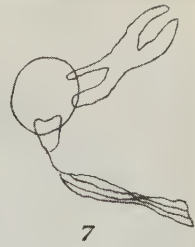
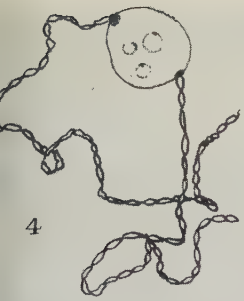
Fig. 51. Zygotene stage showing the attachment of three bivalents by their terminal satellites to the compound nucleolus, as indicated by arrows.

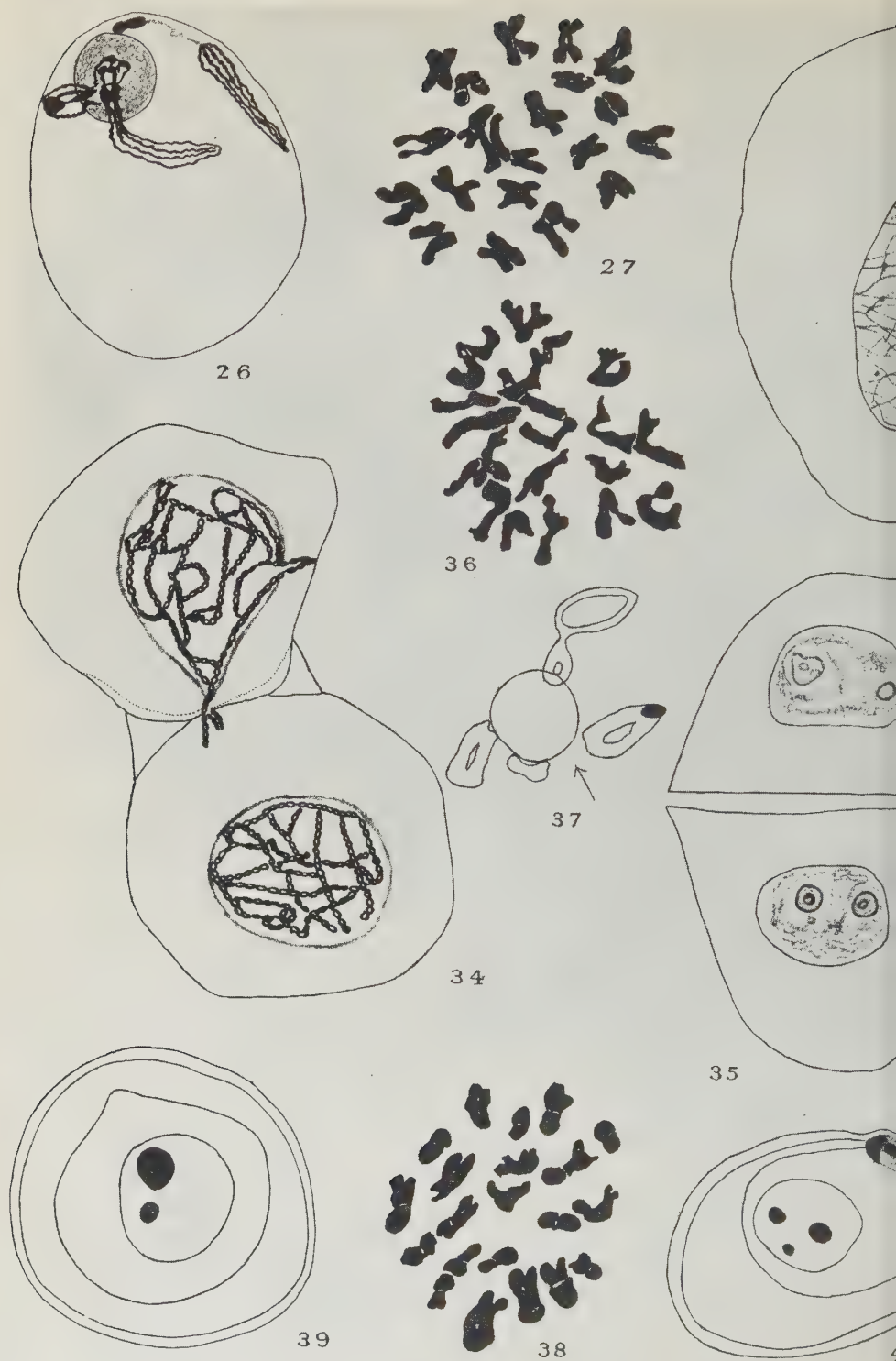
Fig. 52. Diakinesis, showing the delicate thread (marked by an arrow) connecting the body of the bivalent with the satellite attached to the nucleolus.

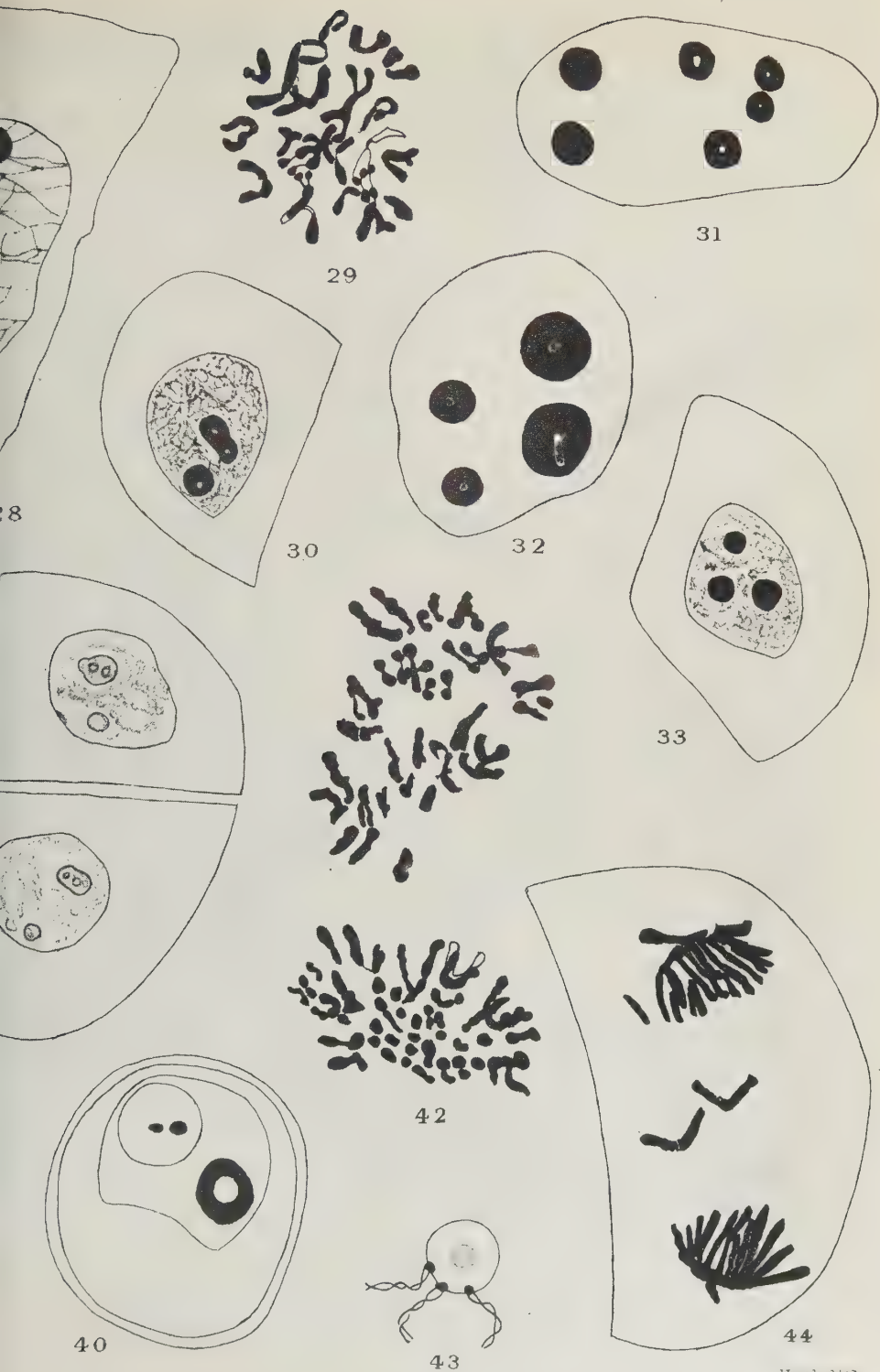
Figs. 53 and 54. Show the ears of *T. vulgare* and of *T. dicoccum* respectively.

Figs. 55 to 67. The different segregates in about their 23rd generation from the hybrid *T. vulgare* × *T. dicoccum*. Segregates Nos. 55 and 56 resemble very much the maternal parent *T. vulgare*, in respect of awn and ear characters, whereas segregates Nos. 63, 65, 66, and 67 resemble very much the paternal parent, *T. dicoccum*, in respect of awn and ear characters.









The Nitrogen Requirements of *Nematospora gossypii* in Synthetic Media

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ATTENTION was first drawn to the nutritional peculiarities of *Nematospora gossypii* by Farries and Bell (1930), who pointed out that this organism is unable to grow in artificial media where the supply of nitrogen is in the form of hydrolysates of highly purified proteins (that is, amino-acid mixtures) except in the presence of small amounts of an 'accessory factor' of the 'bios' type. This accessory factor was shown to be present in crude proteins such as egg-white, and in commercial peptone. Further work established the fact that the accessory factor is of complex nature, consisting of at least two components, one of which is meso-inositol (Buston and Pramanik, 1931), while the second remains unidentified. Recent work suggests that the second component may be the crystalline 'biotin' of Kögl (1937) and Kögl and Tonnies (1936).

In the investigations on the nature of the accessory factors the nitrogen necessary for the organism was usually supplied in the form of acid-hydrolysed gelatin; however, a more detailed study of the nutritional requirements of the fungus led to the use of simpler nitrogenous substances. This seemed desirable in view of the fact that protein hydrolysates may contain traces of unidentified compounds having a considerable influence on the growth of micro-organisms; thus, it has been pointed out by Knight and Fildes (1933) that gelatin, which has usually been regarded as lacking in tryptophan, actually contains enough of this amino-acid to permit the growth of *Bacillus (Clostridium) sporogenes*, for which it is an essential. In order, therefore, to define the simplest artificial medium suitable for the growth of *N. gossypii*, the ability of the organism to utilize simple nitrogenous compounds such as ammonium salts, amino-acids, &c., was investigated; some of the more interesting observations are recorded here.

EXPERIMENTAL

1. General experimental method.

Details of the method of culture have been recorded in previous papers (Buston and Kasinathan, 1933; Buston and Pramanik, 1931; Farries and Bell,

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1930). The basal medium consisted, as before, of: glucose, 2 per cent.; K_2HPO_4 , 0.5 per cent.; $MgSO_4 \cdot 7H_2O$, 0.25 per cent.; agar, 2 per cent.; inositol, 0.01 per cent.; 'factor II', a concentrate prepared from lentils by the method of Buston and Kasinathan (1933) active in doses of 7–8 mg. per 100 c.c. of medium. Nitrogen, from whatever source, was introduced to give a concentration equivalent to 0.3 per cent. N. Since no concentrate of the second accessory factor has yet been obtained free from nitrogen, allowance must be made for the quantity of nitrogen introduced into the medium from such concentrates. In the experiments here recorded, the amount of nitrogen so introduced was approximately 0.9 mg. per 100 c.c. of medium, i.e. 0.3 per cent. of the total nitrogen. Even so small an amount as this may, of course, be the means of introducing the necessary amount of an essential nitrogenous constituent, although this is unlikely to be one of the amino-acids shown in these experiments to have a stimulant action.

The standard incubation was at 30° C. for seven days. Under these conditions, with hydrolysed gelatin as the source of nitrogen, the dry weight of mycelium from pairs of 3-inch Petri dishes was 270–80 mg., and this is taken as the standard growth.

2. *Growth on amino-acids.*

The amino-acids used were purified as far as possible by recrystallization, and, where available, were of synthetic origin. Tests were made with a considerable range of amino-acids, separately and mixed in various proportions. The results are not recorded in full, but typical examples are given in Table I.

It was apparent from results such as those in Table I that even so simple a mixture of amino-acids as glycine and aspartic acid was adequate as a source of nitrogen. On the other hand, none of the amino-acids examined was individually adequate for full growth, although aspartic acid and some samples of glutamic acid gave growths approaching the full amount. Some difficulty was experienced in the case of glutamic acid, different samples of which differed in their behaviour (see Table I). It is possible that some very active growth-stimulant is associated with preparations of the acid from natural sources, but no sample of the synthetic acid was available to test this point. It may be mentioned that other workers have had similar experiences with glutamic acid (Mueller and Kapnick, 1935). In the case of aspartic acid no such variability was noted.

3. *Growth on ammonium salts.*

Salts of both inorganic and organic acids were investigated, and in the great majority of cases gave but poor growth; yields of the dry mycelium were usually of the order of 50 mg., i.e. about one-fifth of the standard. In contrast with the majority of ammonium salts, ammonium aspartate gave growth practically equal to that on hydrolysed gelatin. Table II records typical results.

It may be pointed out that if less active concentrates of the second accessory factor are used quite good growth may be obtained with ammonium salts or amino-acids which give but poor growth with the purer concentrates.

TABLE I
Source of Nitrogen and its Percentage of Total Nitrogen

Glycine.	Leucine.	Aspartic acid.	Proline.	Histidine.	Growth (mg.).
30	20	28	20	2	277
40	20	38	—	2	271
50	—	50	—	—	240
50	—	50*	—	—	128
75	—	25	—	—	275
100	—	—	—	—	55
—	—	100	—	—	230
Glutamic acid.					
50	—	50	—	—	235
—	—	100	—	—	215
—	—	100†	—	—	164

* *dl*-aspartic acid.

† Second sample (Fraenkel and Landau).

TABLE II

Source of nitrogen.	Growth (mg.).
Ammonium sulphate	63
„ „ plus CaCO ₃	53
„ nitrate	50
„ chloride	0
„ tartrate	62
„ lactate	60
„ pyruvate	30
„ aspartate	275
„ <i>dl</i> -aspartate	275

This may be explained as due to the presence in the crude concentrates of small but active amounts of, for instance, aspartic acid (see below, section 6).

Further experiments showed that good growth was obtained when the nitrogen supply was partly in the form of an ammonium salt and partly in the form of aspartic acid—that is, the otherwise unavailable nitrogen of the ammonium salt was rendered available in the presence of the aspartic acid. This may be interpreted on the view that the organism is able to transform ammonia into some of the more complex nitrogenous derivatives, but not into aspartic acid which is necessary either in itself or as a stage in the production of some further compound. So far as was found, no other amino-acid behaved similarly.

Table III shows the effect of adding certain amino-acids to media containing ammonium salts (see also Table V).

4. *Growth of asparagin.*

Farries and Bell (loc cit.) noted that, while asparagin alone, in the absence of accessory factors, was unable to support growth of *N. gossypii*, yet in the presence of peptone, 'egg-extract', or 'casein-extract' there was some utilization of this amide. Further tests with inositol and the lentil concentrate, as

TABLE III

Ammonium salt.	% of total N.	Amino-acid.	% of total N.	Growth (mg.).
Nitrate . . .	50	Glycine . . .	50	40-50
" . . .	50	Alanine . . .	50	40-50
" . . .	75	Leucine . . .	25	40-50
" . . .	50	Aspartic acid . . .	50	240
" . . .	50	Glutamic acid . . .	50	85
Sulphate . . .	50	Glycine . . .	50	55
" . . .	50	Aspartic acid . . .	50	235
" . . .	50	Glutamic acid . . .	50	120
Tartrate . . .	67	Aspartic acid . . .	33	190

used in the present series of experiments, showed that asparagin alone formed a perfectly suitable source of nitrogen, growth taking place at the normal rate and to the full extent, as on gelatin hydrolysates; the only observable difference being that on the asparagin media the yellow pigment produced on gelatin was lacking. Thus, in the presence of adequate supplies of the accessory factors, the organism is able to synthesize all its nitrogen compounds from asparagin, as from glycine and aspartic acid (Table I).

5. *The effect of β -alanine.*

Williams and Rohrman (1936) have observed, in the case of yeast, that β -alanine in a concentration of 17 per 12 c.c. of medium has a markedly stimulating action on cell-multiplication. This effect was not seen in the presence of asparagin; on the other hand, in the presence of aspartic acid, the action of β -alanine was very greatly enhanced. Stimulation of yeast-growth by β -alanine has also been observed by Kögl (1936) and others. It has also been noted that with higher concentrations of β -alanine the effect is not found, and Nielsen (1936) has shown that this amino-acid cannot function as the sole source of nitrogen for yeast.

Tests with *N. gossypii* showed that, in ammonium nitrate or tartrate media, β -alanine in minute concentrations had also a stimulant action on growth; the effect was by no means so large as with yeast, and in larger doses growth of the fungus was actually inhibited (Table IV). In the presence of aspartic acid, the addition of β -alanine in doses of 1-10 mg. gave no increase in growth.

6. *The influence of aspartic acid and asparagin.*

From the results given in Tables I-III it was seen that ammonium aspartate is especially suitable as a source of nitrogen for *N. gossypii*; asparagin is

equally effective (section 4), and aspartic acid itself is but little inferior; β -alanine also is shown to have a stimulant action on growth. Ackermann (1911) showed that some bacteria were able to convert aspartic acid into β -alanine by decarboxylation; and it seemed possible that *N. gossypii* could effect the same degradation during growth, the small amounts of β -alanine

TABLE IV

Ammonium nitrate, mg. per 100 c.c. of medium.	β -alanine.		Growth (mg.).
	mg. per 100 c.c.	% of total nitrogen.	
857	0.1	0.005	50-5
857	1	0.05	90
853	10	0.52	175-80
838	50	2.60	60
812	100	5.20	55

thus continuously produced then exerting their stimulant action. This would account for the special activity of aspartic acid and its derivatives, and also for the fact that addition of β -alanine had no effect on a medium already containing aspartic acid. If this view is correct it should be possible for the fungus to grow on ammonium salt media in the presence of amounts of aspartic acid sufficient only to produce the essential quantities of β -alanine.

Tests with media containing ammonium nitrate or tartrate, in the presence of aspartic acid or asparagin, showed that these substances in relatively low concentrations had a stimulant effect rather greater than that of β -alanine itself (Table V).

Since both the aspartic acid (the *l*-acid, from protein hydrolysates) and the asparagin used in the work were of natural origin, further tests were made with a sample of synthetic *dl*-aspartic acid (Hofmann-La Roche), the results of which are also given in Table V. These indicate that the stimulant effect is definitely due to aspartic acid itself, and not to an unknown contaminant derived from the natural source of the *l*-aspartic acid. It was observed that for the lower concentrations of the synthetic acid its activity was roughly one-half that of the *l*-isomer. A sample of the *l*-acid, prepared from the *dl*-mixture by the method of Fischer (1899), was found to have an activity equal to that of the natural *l*-aspartic acid (100 mg. acid per 100 c.c. medium gave a growth of 220-5 mg. dry wt.).

DISCUSSION

The special role played by aspartic acid in the nutrition of some micro-organisms has been indicated by previous workers; thus, Williams and Rohrman (loc. cit.) found that the acid, even in absence of β -alanine, gave a definitely increased yeast crop, while Mosher et al. (1936) states that it is 'very helpful' though not indispensable in the nutrition of *Tricophyton interdigitale*. The case of *N. gossypii* may, therefore, be regarded as a further instance of this

special role. The important position of the related amide, asparagin, in the nitrogenous metabolism of the higher plants needs no emphasis.

The suggestion that aspartic acid acts as a precursor of β -alanine seems feasible, but the possibility that it may have other functions is not excluded.

TABLE V

Ammonium nitrate, mg. per 100 c.c.	Aspartic acid		Growth (mg.).
	mg. per 100 c.c.	% of total nitrogen.	
855	10	0.35	90
849	25	0.87	112
842	50	1.75	190
827	100	3.50	220
812	150	5.25	244
797	200	7.00	257
782	250	8.75	235
Ammonium tartrate.			
1960	10	0.35	82
1940	50	1.75	140
1900	100	3.50	193
1860	150	5.25	230
Ammonium nitrate. Asparagin.			
855	5	0.35	40-50
853	10	0.70	90
827	50	3.52	130
812	75	5.28	158
796	100	7.04	180
766	150	10.56	205
736	200	14.08	252
Ammonium aspartate.			
832	50	2.92	130
<i>dl</i> -Aspartic acid.			
855	10	0.35	40-50
849	25	0.87	66
842	50	1.75	100
827	100	3.50	144
812	150	5.25	193
797	200	7.00	240

That aspartic acid is more active than β -alanine suggests that the latter is most effective when produced continuously at a concentration approaching the optimum for growth-stimulation, thus avoiding the inhibiting effect of excessive concentrations. Both *d*- and *l*-aspartic acid would give β -alanine (which is inactive) by decarboxylation, but the lower growth-stimulating activity of the *dl*-acid as compared with the *l*-isomer could be explained on the grounds that *N. gossypii* does not possess the enzyme system necessary to effect the degradation of the *d*-acid, which does not occur among natural products.

The fact that β -alanine and aspartic acid are most efficient for yeast when in combination is not easily explained, and suggests that for yeast, at any rate, aspartic acid has some function other than the production of β -alanine.

SUMMARY

In the presence of inositol (10 mg. per 100 c.c. of medium) and of sufficient amounts of the 'second accessory factor' (from lentils), *Nematospora gossypii* is able to grow on a medium whose sole nitrogenous constituent is asparagin, or ammonium aspartate; simple mixtures of amino-acids including aspartic acid are also suitable, and tryptophan is not essential.

Ammonium salts of inorganic acids and of most organic acids are not adequate as a source of nitrogen, but in the presence of β -alanine (1-10 mg. per 100 c.c.) moderate growth is obtained; *L*-aspartic acid (100 mg. per 100 c.c. medium, i.e. providing 3.50 per cent. of the total nitrogen) stimulates growth on ammonium salt media to the extent of about 90 per cent. of the standard growth on gelatin hydrolysates or on asparagin.

We wish to thank Professor W. Brown for facilities offered in the Department of Plant Pathology.

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The Cytology and Development of *Phyllactinia corylea* Lév.

BY

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With Plates XVI and XVII and twenty-seven Figures in the Text

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INTRODUCTION

TO follow the behaviour of the nuclei in a fungus which consists of uninucleate cells would seem at first sight a task in which great precision could be attained; in fact, much greater precision might be hoped for than where large multinucleate cells are under observation. The presence of uninucleate cells together with a certain accumulation of evidence in favour of the possession of a functional antheridium led to a reinvestigation of several members of the Erysiphales. It was hoped that a careful study of the chromosome number at every possible stage in the life-history would furnish incontrovertible proof or disproof of the fusion of male and female nuclei in the oogonium, in this very small natural group of fungi.

The occurrence of such a fusion has been described, contradicted, and redescribed by many workers during the last fifty years. The nuclear divisions in the ascus have been figured and described, more or less incompletely, several times, but no serious attempt has been made to give a complete account of the chromosome number throughout development for any one member of the group.

In order to make clear the general position and the scope of the present work, brief references to the past history of studies of the group as a whole must be given.

The main relevant point from de Bary's (1887) descriptions is that, in all the plants examined by him, the antheridium lay closely pressed to the tip of the oogonium. This fact suggested to him that fertilization took place.

Harper's work (1895, 1896, 1897) on *Erysiphe* and *Sphaerotheca* confirms and extends de Bary's account. For both these plants Harper described and figured the passage of the male nucleus from the antheridium into the oogonium and the fusion there of the two nuclei. In his work on *Phyllactinia corylea* (1905) the account of fertilization and the growth of the perithecium was augmented by a study of the nuclear divisions in the ascus. A short account of fertilization and the first few cell-divisions in *Sphaerotheca humuli* was given by Blackman and Fraser (1905) which confirms Harper's work on this plant.

All the plants examined by Dangeard (1896) and his successors (Moreau and Moreau, 1929; Eftimu and Kharbush, 1932; Raymond, 1933) were described as apogamous. The only fusion seen by them was that in the ascus. An apogamous form of *Sphaerotheca* was examined by Winge (1911). He described a degenerating nucleus in the antheridium at the time when the oogonium had continued its development into a binucleate cell.

Yet another variation of the story was given by Bezssonov (1912) for *Sphaerotheca mors uvae*. He stated that after the antheridial cell was formed its nucleus divided. One of the daughter nuclei passed into the oogonium and was said to fuse with the female nucleus; the other nucleus remained behind in the antheridium and degenerated there. Such a division has not been described for any other Erysiphaceous fungus and, although it offers an ingenious explanation of the two conflicting accounts for *Sphaerotheca*, it must remain open to very considerable doubt.

The account given by Allen (1936) of *Erysiphe polygoni* presents a life-history at variance with the descriptions of all the earlier workers. She rejected the idea that definite sexual branches were formed, and regarded as the perithecial initial any irregular hyphal twist in the mycelium. She suggested that at every such initial a nuclear migration had taken place, and that, as growth continued, 'cell fusion is followed by incomplete closure of the septa, resulting in the formation of chains of communicating cells or mazes. . . . These cells, evidently sporophytic in character, contain from one to several nuclei.' The story is further complicated by her statement that the central region of the perithecium degenerated and that the ascogenous hyphae arose from cells of the 'sporophytic' sheath.

Closely connected with the question of sexuality and apogamy is that of the occurrence of heterothallism within the group. The report that *Erysiphe cichoracearum* (Yarwood, 1934) is heterothallic rests mainly on field observations as to the position of the perithecia in the fungal colonies. They are said to be found only at the intersection of two neighbouring colonies. This observation has in support of it a small body of experimental data from single spore cultures (Yarwood, 1934). With regard to *Sphaerotheca*, however, work by Homma (1934) leads to the conclusion that this fungus is sexually perfect and homothallic.

It will be seen from this very brief summary that species of *Sphaerotheca* have been said to be both sexually perfect and apogamous; *Erysiphe* has been

described as sexual, as apogamous, and as apogamous with the further complication of nuclear migrations from hypha to hypha; *Phyllactinia* is said to possess a sexual fusion, but this is not borne out entirely by the chromosome counts in the ascus.

The present paper is limited to an account of *P. corylea*. Several other species, *S. humuli*, *S. humuli* var. *fuliginea*, *E. polygoni*, and *Uncinula aceris* are under investigation, but it has proved unexpectedly difficult to obtain all the necessary nuclear divisions to complete the life-histories in these species. *Phyllactinia*, however, has given a sufficiently complete range of stages to justify separate publication.

MATERIAL AND METHODS

P. corylea occurs commonly on *Corylus avellana* in the southern counties of England. The form on this host was used exclusively for the present work. Fixations were made in the field during three successive seasons from one locality in Hampshire, but the consistency of the results obtained from this material was checked by collections from other places all within a thirty-mile radius of the main locality.

The fixatives used were all the usual variations of the chromic, osmic, acetic, acid mixtures, including Flemming's weak and half-strength fluids and the Merton 2BD and 2BE fixatives (La Cour, 1931). All these fixatives were tried, using the indicated quantity of osmic acid and with this quantity halved and doubled. In addition to the fixatives containing osmic acid, various modifications of Navashin's chromic acetic formalin mixture were used. With all the fixatives pretreatment with Carnoy and with absolute alcohol was tried. The best results were obtained with 2BD without pretreatment. Even this caused slight shrinkage of the cytoplasm which no change in the original formula would eliminate, but the nuclei were excellently fixed. With all the fixatives pretreatment was detrimental; the use of Carnoy, in particular, plasmolysed the cells quite fatally.

For all fixings the use of an exhaust pump was essential. Even after the most vigorous pumping, a little air sometimes remained round a few of the perithecia; this resulted in incomplete penetration by the fixative, and these perithecia could always be picked out in the finished preparations by their affinity for the stain. Sometimes the whole perithecium remained persistently black, in spite of prolonged treatment with iron alum; more often only the fertile region retained the stain. Such blackened perithecia formed only a very small proportion of all those examined. Apart from their staining capacity they seemed normal in every way, and there was nothing to suggest that a degeneration of the central fertile region of the perithecium at any time formed an integral step in the life-history of the plant (see p. 399).

The fixed material was washed, dehydrated, and embedded in the usual way. During the embedding process the material was passed through several changes of melted wax to ensure complete penetration of the leaf tissue by

pure wax. If this precaution was not taken the leaf sections fell off the slide during staining, dragging the fungal hyphae with them. Most of the material was stained in Heidenhain's iron haematoxylin, the sections being mordanted in 4 per cent. iron alum and de-stained in 8 per cent. alum containing a etic acid (Gwynne-Vaughan and Barnes, 1927). For nuclear divisions in the ascus a modification of Newton's gentian violet was used (Manton, 1930).

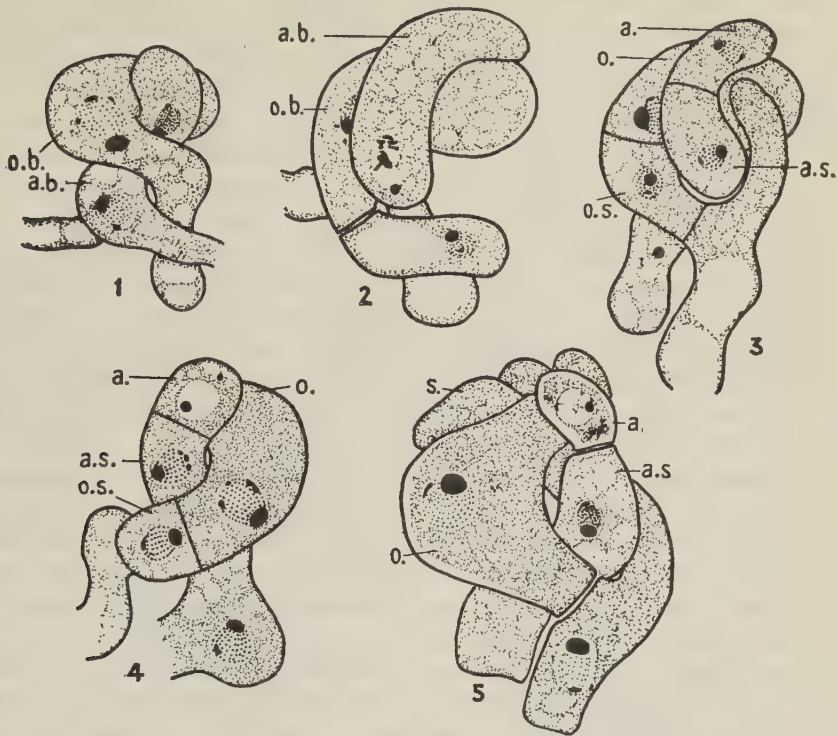
The main difficulty with the work lay in the scarcity of nuclear divisions in the young perithecia. There was no lack of divisions in the asci giving beautiful nuclear figures, but mitoses in the early stages of development were completely absent from the first season's fixings, only rarely to be found in the second season, and but slightly more plentiful in the third. Two-hourly fixings over a period of twenty-four hours and daily fixings for a week in the third season produced the bare minimum of dividing nuclei necessary to complete the chromosome counts. The three batches of fixed material which contained these dividing nuclei were all fixed between 10.30 a.m. and 3 p.m. on days which were sunny but not hot, following a day or night of rain.

DEVELOPMENT OF THE PERITHECIUM

Young reproductive branches begin to appear at the centre of the circular patches of mycelium on the Hazel leaves during the last week in August. The two kinds of sexual branches are at first very much alike, but are always borne on different hyphae and seem to arise where two actively growing filaments have come into contact (cf. *Mucorales*, Blakeslee, 1904). In no case has anything in the way of protoplasmic connexions or passage of nuclei been seen at this stage.

The young sexual branches grow up at right angles to the substratum and early in their development become coiled about each other (Pl. XVI, Fig. 1). Each branch contains a nucleus, and, even before these nuclei divide, the two branches have become markedly different from one another. The female branch is fatter than the male, is considerably more curved (Text-fig. 1), and may be bent over so that the greater part of its length lies parallel to the leaf surface. The male branch is only slightly curved, so that its tip lies across the end of the female branch. Each sexual branch soon becomes cut off from its parent hypha by a transverse wall (Text-fig. 2). The nucleus in each branch now divides and the daughter nuclei in both antheridial and oogonial hyphae become separated from each other by a cell wall (Text-figs. 2 and 3). In the antheridial branch the upper cell, the antheridium, is small, only slightly longer than broad, and has a correspondingly small nucleus; the rest of the original branch forms the long stalk cell (Pl. XVI, Fig. 2). In the oogonial cell the proportions are quite different; the upper cell, the oogonium, is by far the larger of the two and contains a large nucleus, while the stalk cell is small and inconspicuous. The oogonium is much stouter than the antheridium and has a bluntly rounded tip across which the antheridium lies (Text-fig. 4). Sheath hyphae now begin to grow out from the stalk cell of the oogonium

(Pl. XVI, Fig. 2) and sometimes from the parent hyphae also (Text-fig. 3). Meanwhile, as the sheath hyphae begin to appear, a change can be seen in the antheridial nucleus (Pl. XVI, Fig. 3, and Text-figs. 4 and 5). The nucleolus remains unchanged, but the rather granular chromatin decreases in quantity



TEXT-FIGS. 1-5. Uncut sexual branches. Fig. 1. Young sex branches, the two sexes differentiated but not cut off from the parent hyphae. Fig. 2. After formation of basal septa, nucleus in the antheridial branch in division. Fig. 3. Antheridial and oogonial cells with stalk cells cut off, sheath formation beginning, male nucleus unchanged. Fig. 4. Antheridial nucleus degenerating, no signs of sheath. Fig. 5. Oogonial cell enlarged and further degeneration of antheridial nucleus. *ab.* antheridial branch, *ob.* oogonial branch, *as.* antheridial stalk, *os.* oogonial stalk, *o.* oogonium, *a.* antheridium s. sheath. All $\times 1,700$.

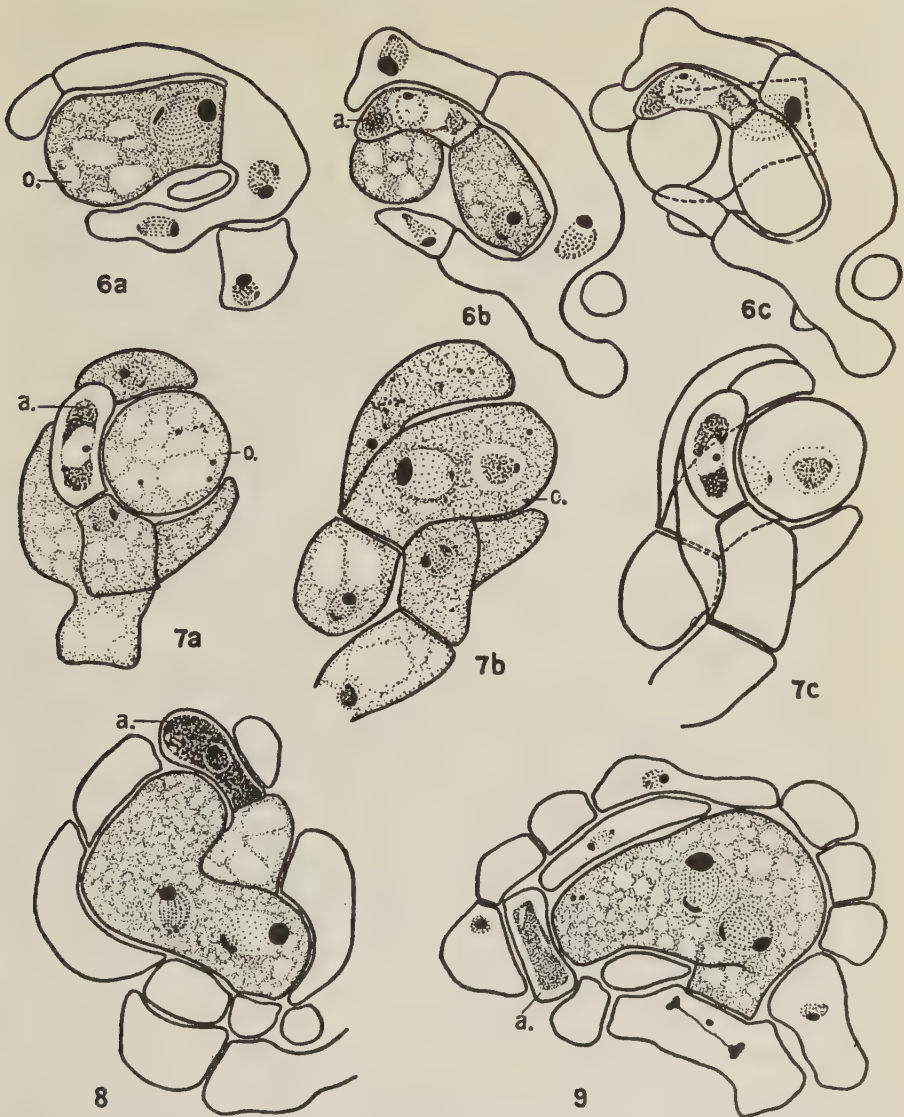
and becomes scattered irregularly about the slightly enlarged nuclear area so that the whole appearance of the nucleus suggests degeneration (compare Text-figs. 3 and 4, also Pl. XVI, Figs. 2 and 3). These two events, the degeneration of the antheridial nucleus and the beginning of sheath formation, do not always take place in the same order, for some examples have been found where nuclear degeneration has preceded all signs of sheath formation (Text-fig. 4) (cf. Dangeard on *Sphaerotheca*, Figs. 8 and 10).

Sheath development and degeneration of the antheridial nucleus continue while the oogonium elongates and its nucleus increases in size and chromatin content. As the oogonium grows it becomes more curved because the

antheridium remains during the whole time closely pressed to the oogonial tip, and the male branch itself does not increase in length. The stalk cell of the antheridium occasionally contributes hyphae to the sheath, but it is always easy to distinguish between sheath and antheridial stalk, since the latter is considerably thicker than any of the sheath branches. Each sheath hypha receives a nucleus from the stalk from which it arises. The nucleus of the stalk cell is constantly dividing to provide nuclei for the sheath. The sheath hypha elongates, its nucleus divides, and the upper of the two nuclei may then be cut off from its sister by a cell wall. The lower nucleus remains in the basal region of the hypha, which may still be in open communication with the stalk cell. The stalk cell, which gives rise to about six primary sheath hyphae, also contains a nucleus (Text-fig. 6*a*). This transitory condition gives the effect of an irregularly shaped binucleate cell, or, if the stalk-cell nucleus has already divided again, a multinucleate cell. All the sheath hyphae are ultimately cut off from the stalk cell. These primary sheath hyphae are developed separately from the oogonial cell and are never in communication with it. Some of the first-formed sheath hyphae grow up perpendicularly to the leaf surface, while others curve so as to cross the sexual branches obliquely. The outer curve of the oogonium is the last to be covered by sheath.

The oogonium has reached its full size by the time it has become completely enclosed (Text-fig. 6*a*, *b*, and *c*). The antheridium, still pressed to the tip of the oogonium, is changed but recognizable. The nucleus is visible as an almost colourless sphere, empty except for the nucleolus, and the cytoplasm has begun to show a marked affinity for the stain, so that the cell stands out from the colourless cells of the sheath (Text-fig. 6*b*). In no case out of the hundred or so examined has any communication between the antheridium and the oogonium been seen; the antheridial wall, although apparently firmly stuck to the oogonium, remains intact, and nothing has been seen to suggest that the nucleus or any of the cell contents passes into the oogonium.

Nevertheless, what seems to be the next stage in development does present a rather startling appearance. The sheath is still growing rapidly and nuclear divisions are fairly frequently encountered here (Text-fig. 7*b*). The antheridium with its stalk is still visible, the antheridium itself being in a fairly advanced state of degeneration, with dark cytoplasm, a degenerating nucleus, and a much swollen, transparent wall (Text-fig. 7*a*). The oogonium presents the most striking difference from that described above as the preceding condition. It is binucleate, and what is more, the two nuclei are different in appearance and size. The oogonium is more curved than before and in the bend near the stalk lies one nucleus. This nucleus possesses a very large nucleolus, a conspicuous lateral granule, and a quantity of granular chromatin which completely fills the nuclear area. In the upper part of the oogonium where it curves towards the antheridium is a second nucleus. In size it is slightly smaller than the one already described, although the actual volumes of the two nuclei are not vastly different. The real difference lies in the dis-



TEXT-FIGS. 6-9. Fig. 6. Young perithecium cut into two portions. (a) Uninucleate oogonium with stalk cell and sheath. (b) Antheridium with degenerating nucleus and cytoplasm, stalk still visible. (c) Reconstruction of the two sections. Fig. 7. Slightly older perithecium from two sections. (a) Tip of oogonium with antheridium attached, male nucleus visible but degenerating. (b) Binucleate oogonium, nuclei unequal in size. (c) Reconstruction of the two sections. Fig. 8. Binucleate oogonium, nuclei more nearly equal in size and degenerating antheridial cell; the sheath has been cut from each side of the oogonium. Fig. 9. Binucleate oogonium, nuclei equal in size, antheridium much changed. a. antheridium, o. oogonium. All $\times 1,700$.

position of the chromatin and the size of the nucleoli. In the smaller nucleus the chromatin is concentrated towards the centre of the nuclear area, leaving

a clear space between it and the cytoplasm of the oogonium. The nucleolus and the lateral granule are smaller than those of the nucleus near the stalk (Text-fig. 7*a*, *b*, and *c*).

Since the antheridium still contains a nucleus it seems that these two nuclei must be the result of a nuclear division in the oogonium, but unfortunately the dividing nucleus has not been seen. Nuclear divisions are at all times rarely found in the young perithecia, except in the sheath; the division preceding the missing one has only been found three times and the division immediately following only four times in all. The whole question of these nuclei will be referred to later (p. 397).

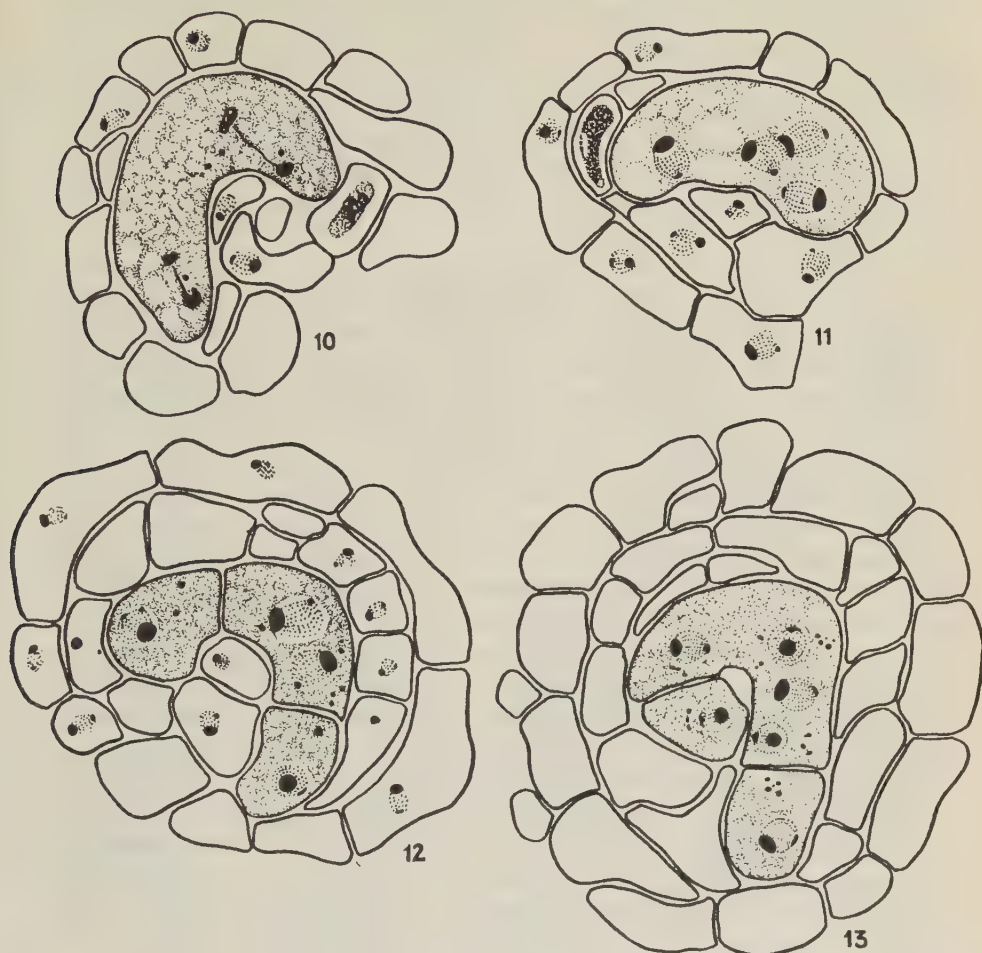
Whatever the origin of the two unequal nuclei, their fate is certain. They do not fuse. The smaller of the two increases gradually in size (Text-fig. 8 and Pl. XVI, Fig. 4) and in the amount of its contents until it becomes almost exactly like the other (Text-fig. 9). Meanwhile the antheridium has become more and more opaque, the nucleus is at first still visible (Text-fig. 8 and Pl. XVI, Fig. 4), but later, as degeneration continues, the whole cell is compressed by the surrounding sheath (Text-fig. 9). The sheath hyphae have by this time become septate throughout their length and the sheath now consists of a layer of cells, one cell thick on the outer curved side of the oogonium, but more than one cell thick on the inner curve (Text-fig. 9).

The two nuclei in the oogonium now divide simultaneously, the spindles being placed obliquely one at each end of the cell (Text-fig. 10). The crushed antheridium is still visible near the tip of the oogonium, showing up as a flattened dark-coloured cell with a much swollen wall. The four daughter nuclei, when division is complete, lie more or less in a row, in the curve of the non-septate oogonium (Text-fig. 11). Two cell walls soon appear between the nuclei forming across the oogonium at right angles to the position of the spindles. This results in a row of three cells. The nuclei, because the septa are laid down across the spindles, are always arranged in the same way. The top cell of the row contains one nucleus, the middle cell contains two nuclei, one from each of the two original nuclei, and the basal cell the remaining one of the four (Text-fig. 12). When first formed, the transverse walls are very thin and may be easily overlooked, but they become thicker and more obvious after a while. In all the perithecia of this age in *Phyllactinia* this three-celled condition is the rule. Even the cut examples at this stage could all be reconstructed on the three-celled plan.

Of the three cells now forming the fertile branch, the tip cell takes no further important part in the development and can often be seen intact at a later stage (Text-fig. 13). The basal cell of the row functions as a stalk cell; it usually remains undivided and puts up slender hyphae which form an inner layer of sheath cells; occasionally it divides by a transverse wall forming two uninucleate cells, both of which may give rise to inner sheath. Towards the end of the season this tendency to divide becomes more marked and young perithecia with as many as five uninucleate cells, supporting the binucleate

cell, have been seen. It is possible that such a perithecium never reaches maturity.

The main business of the further development of the fertile region of the



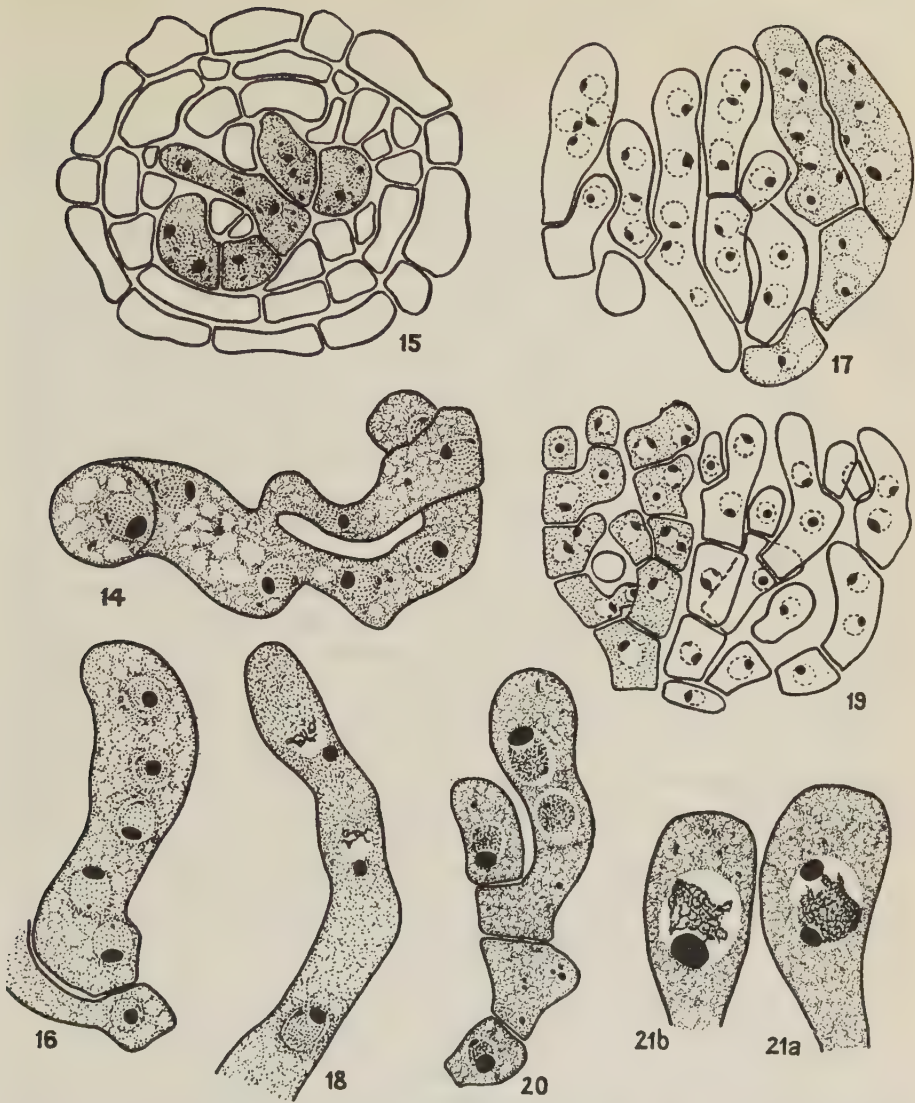
TEXT-FIGS. 10-13. Developing fertile branch. Fig. 10. Nuclear division in binucleate oogonium, nuclei in telophase, antheridium still visible. Fig. 11. Oogonium with four nuclei seen from the top of the curved cell, antheridium still visible. Fig. 12. Three-celled fertile branch seen from the side, branch uncut and all the nuclei visible. Fig. 13. Three-celled fertile branch after nuclear division in the central cell, tip and basal cell still undivided; reconstructed from two sections. In all these figures the cytoplasm has been shown only in the central cells of the perithecium. All $\times 1,700$.

perithecium devolves upon the binucleate cell. It continues to elongate and the nuclei divide so that at first a quadri-nucleate cell is formed (Text-fig. 13). These four nuclei divide at least once more, so that a curved multinucleate cell is formed. Active growth of the whole perithecium accompanies these nuclear divisions, the sheath being augmented by the inner layer from the

basal cell and as the fertile cell elongates it is forced to curve because of the pressure from the sheath. At the same time short branches grow out from the upper side of the multinucleate fertile cell, away from the leaf surface, and some of the nuclei pass into them (Text-fig. 14). These branches are the young ascogenous hyphae. As the ascogenous hyphae appear the multinucleate cell from which they arise becomes cut up by cross walls into a number of irregular portions. These stages have often to be reconstructed from several sections, so that it is impossible to settle the exact distribution of the nuclei. The fertile branch is always curved, and some of the cells are cut. Portions containing one, two, or more nuclei can be seen, but these are not always complete cells (Text-fig. 15).

The ascogenous hyphae increase in length and branch. At first they stand erect and perpendicular to the leaf surface. A little later, as growth continues, they may be temporarily pushed sideways by the surrounding sheath, which, although constantly increasing, does not always keep pace with the rapid growth of the central region. At this time any sheath cell may contain more than one nucleus, if cell division has not immediately followed nuclear division. After a while the sheath appears to catch up with the growth in length of the ascogenous hyphae, which once more stand erect. It is easy here, as at all times, to distinguish between the sheath and the central fertile branches, since the latter are always thicker and contain more cytoplasm and larger nuclei than the sheath cells. Up to this time the ascogenous hyphae are branched and multinucleate. They are also non-septate, but occasionally where a branching has taken place a transverse wall is formed. Each branch contains four or more nuclei (Text-figs. 16 and 17). The original fertile cells from which the ascogenous hyphae arose disappear as the system becomes increasingly branched, but it can still be seen that the main branches have all arisen very close together, consistent with their origin from two or three nearly related cells. Only the branches present in one thick section are shown in the figure, but the whole system is branched in all directions. In obliquely cut sections the ascogenous hyphae present a bewildering mass of multinucleate, binucleate, and uninucleate cut portions which are quite unintelligible, and only those perithecia which have not been disturbed from their original position on the leaf and have been cut exactly through their attachment to the leaf show the regular arrangement of the branches.

The nuclei in the non-septate ascogenous hyphae now divide. These divisions are not simultaneous in all the branches and have only been seen a few times. Two nuclei are shown in prophase in Text-fig. 18, and a few telophases have also been found besides the one countable metaphase to be referred to later. After nuclear division, cell walls are formed in the ascogenous hyphae across the spindles, and this results in a row of cells in each branch with a uninucleate tip cell, a varying number of binucleate cells occupying the middle region of the branch, and a uninucleate basal cell (Text-fig. 19). This arrangement of the nuclei is quite consistent and has been seen in many



TEXT-FIGS. 14-21. Development of the ascogenous hyphae. Fig. 14. Central fertile region of the perithecium with two young ascogenous hyphae. The perithecium was cut into three; the other two sections each contained only sheath and a small uninucleate piece of the fertile branch. Fig. 15. Section of perithecium slightly older than the last, with septa in the fertile branch and young ascogenous hyphae. Fig. 16. Older multinucleate ascogenous hypha. Fig. 17. Section of perithecium with branched multinucleate ascogenous hyphae, sheath and some of the cytoplasm omitted. Fig. 18. Ascogenous hypha with nuclei in prophase before division. Fig. 19. Section of perithecium with septate ascogenous hyphae, sheath, and some of the cytoplasm omitted. Fig. 20. Origin of young binucleate ascus; the uninucleate tip cell is still visible. Fig. 21. Fusion of nuclei in the ascus. (a) Fusion of lateral granules. (b) Fusion of nucleoli. Figs. 14, 16, 18, 20, and 21. $\times 1,700$. Figs. 15, 17, and 19. $\times 1,100$.

perithecia. As in the three-celled fertile branch already described, the basal cell of any ascogenous hypha may undergo division so that more than one uninucleate cell may be found at the base of the branch.

Almost as soon as the cell walls are formed the topmost binucleate cell begins to push out to the side of the uninucleate tip cell to form a young ascus (Text-figs. 19 and 20). This development may be repeated by the cell immediately below it in the branch, for any of the binucleate cells can become an ascus, but not all of them reach maturity. The average number of binucleate cells in the ascogenous hypha system is thirty-six, and the average number of asci in the mature perithecium is twenty-four. As the asci increase in size they push aside and absorb the remains of the ascogenous hyphae and the inner layers of the sheath so that the centre of the perithecium becomes filled with a group of young asci which, by adjustment in the growth of their bases, now all lie at one level. They are surrounded by a wall of two or three layers of cells and are at first binucleate, but nuclear fusion occurs before the asci are fully grown.

Each nucleus, just before fusion, has a large nuclear area, a conspicuous nucleolus, a small lateral granule, and a mass of irregularly twisted chromatin threads (Pl. XVI, Fig. 9). The nuclear areas of the two nuclei at first only touch and then the limiting membranes run into each other so that a dumb-bell-shaped area is formed, containing two nucleoli and two chromatin masses. Next the chromatin masses touch, and although the two lateral granules fuse the chromatin masses do not seem to mix intimately with each other but remain as two adjacent groups of chromosomes (Text-fig. 21*a*). The nucleoli remain separate a little longer, but finally they also fuse (Pl. XVI, Fig. 10). At the time of fusion all the individual chromosomes cannot be identified: they are in the form of long thin intertwined threads and only some of the ends are visible (Text-fig. 21*b*). At some time after the fusion the chromosomes must pair, since it is followed eventually, after a period of considerable growth, by the appearance of the bivalents ready for the meiotic division. The actual time of pairing cannot be ascertained.

The whole perithecium, after nuclear fusion in the ascus, consists of a wall of cells, some four or five cells thick, and a central group of twenty or more asci. Each ascus contains a nucleus situated near the base where the ascus narrows to a stalk. Further development is mainly an increase in size of all the component parts of the perithecium until they reach their maximum dimensions. The two kinds of appendages begin to grow out from the perithecial wall and, with the appearance of the terminal ones, the nucleus moves up from the base of the ascus to its middle, nearly doubles its size, and proceeds to undergo the usual prophase changes which precede a meiotic division.

CHROMOSOME COUNTS IN THE YOUNG PERITHECIUM

The information obtained from chromosome counts at nuclear division during the growth of the young perithecium is given separately here, rather

than in its apparently more logical position in the preceding account of the development of the ascocarp, in order that its significance shall not be lost in a mass of morphological detail.

Divisions in the early stages of sheath formation are relatively common. In the very best examples ten chromosomes are visible at metaphase (Pl. XVI, Figs. 5 and 6). Many divisions have been seen where nine is the maximum countable at metaphase, but it is fairly certain that in these one of the smaller chromosomes is hidden behind the larger ones (Text-fig. 7b). The spindle is very conspicuous, with a deeply staining granule, the centrosome, at each end. Several anaphases have been seen (Pl. XVI, Fig. 7), and here also nine chromosomes are usually visible; eight can be counted at each end in the figure. These counts by themselves establish the haploid chromosome number for *Phyllactinia* as being in the region of ten.¹

Chromosomes counts have also been made in the developing sex branches, although dividing nuclei are less common here than in the sheath cells. A pair of very young sex branches with the nucleus in the parent hypha of one of them in division is shown in Pl. XVI, Fig. 1. This nuclear division will provide the nucleus for one sexual branch, and eight chromosomes can be seen on the metaphase plate. An early metaphase in the young antheridial branch with eight chromosomes visible is shown in Text-fig. 2, and divisions also with about nine chromosomes at metaphase have been found in young oogonial branches. While these counts do not give an exact haploid number they confirm the more accurate results obtained from divisions in the sheath cells.

Nuclear divisions have also been seen in the ascogenous hyphae, and here again the haploid number is found. One very beautiful metaphase (Pl. XVI, Fig. 8) situated near the base of an ascogenous hypha shows ten chromosomes. Two metaphases in the binucleate oogonium have been seen and these show a maximum of ten, again the haploid number, but these two preparations are not good enough to yield an accurate result and are only quoted as corroborative evidence. The cytological proof that a fusion of nuclei has occurred in the life-history of a fungus such as *Phyllactinia* would be the manifestation of the *diploid* chromosome number at metaphase during nuclear division in the ascogenous hyphae. In this material of *Phyllactinia* only the *haploid* chromosome number has been encountered in the nuclear divisions throughout the development of the young perithecium, including those in the ascogenous hyphae. This evidence, although not overwhelming in its quantity, seems to preclude any suggestion of a nuclear fusion in the oogonium.

It is well to emphasize yet again the relative rarity with which nuclear divisions are found in the young perithecia in fixed material. Only under the optimum conditions of growth at the moment of fixation have they been found

¹ The haploid number of *ten* is established definitely by counts at anaphase in the last nuclear division in the ascus; see next section.

to be present. This result is borne out by the fact that, in all the previous work on the group, not more than a few uncountable metaphases in sheath cells of the young perithecium have been published.

CHROMOSOME COUNTS IN THE ASCUS

In contrast to the position with regard to nuclear divisions in the young perithecium, no difficulty was encountered in obtaining beautifully fixed and stained nuclear figures in the ascus, and the account here is based on many examples.

Heterotype division. The early stages of prophase are considerably prolonged, taking place while the growth of the perithecial appendages is in progress. The spindle develops within the nuclear area while the nucleus is still in prophase, and is at first asymmetrical. The first sign of spindle formation is the division into two of the small lateral granule, the centrosome. These two granules then separate and the spindle appears between them. They continue to move apart and the spindle elongates and straightens out until it reaches across the nuclear area and lies parallel to the long axis of the ascus. By this time the chromosomes are lying in pairs on the spindle. At very early metaphase the ten bivalents still show their characteristic shapes and the nucleolus is unchanged in size (Pl. XVII, Fig. 11). The pairs differ very much in size, there are two very long pairs, six medium-sized pairs, and two very small pairs. At late metaphase, before the pairs finally move apart, they lie closer to each other on the spindle and the pairs themselves are smaller and more compact (Pl. XVII, Fig. 12). In some preparations at this stage one or both of the small pairs may be hidden behind their larger neighbours; both of them are visible in the figured example. An early anaphase where all but two of the pairs have separated, and showing the full diploid number, is seen in Pl. XVII, Fig. 14, while at late anaphase ten chromosomes are arranged near each pole (Pl. XVII, Fig. 15). The nucleus in this figure was situated in a somewhat obliquely cut ascus so that the two anaphase groups were seen nearly from the polar view at considerably different focal levels.

The daughter nuclei, after the first division, lie in the middle of the ascus, each nucleus being conspicuously beaked (Text-fig. 23). These beaks correspond in position to the centrosomes of the previous division and from them the remains of the aster can still be seen radiating out into the cytoplasm. Between the nuclei the remnant of the nucleolus of the fusion nucleus is often visible (Text-fig. 23). During the re-formation of the daughter nuclei the distance between them increases considerably. The lower nucleus, that nearer to the ascus stalk, lies practically in the middle of the ascus, and the upper nucleus is pushed nearly to the ascus tip (Text-fig. 23). The lower nucleus increases in size more rapidly than its sister and is often seen to be approaching metaphase of the homotype division before the upper one is fully grown.

The homotype division follows closely on the heterotype, and both divisions are often to be found in the same perithecium. The spindles are formed for

this division in precisely the same way as for the previous one; that of the lower nucleus lies parallel to the long axis of the ascus and that of the upper nucleus lies at right angles to it and in the ascus tip (Text-fig. 24). The two nuclei



TEXT-FIGS. 22-7. Diagrams showing position of the nuclei during the three nuclear divisions in the ascus. Fig. 22. First metaphase, nucleolus shown as a circle. Fig. 23. Daughter nuclei after first division, remains of nucleolus between the nuclei. Fig. 24. Metaphase of second division, nucleoli shown as circles. Fig. 25. After the second division, the nucleus right at the top of the ascus is the sister of the larger one in the middle. Fig. 26. Third division, large nucleus in the middle of the ascus at anaphase, the other three at the top with chromosomes about to split. Fig. 27. Eight nuclei in ascus, spores beginning to form round the two beaked nuclei in the middle, the other six nuclei without asters or beaks. All $\times 870$.

are shown in metaphase in Pl. XVII, Fig. 17; the upper one, in which the spindle is not fully stretched across the nuclear area, is lagging behind the lower one where the chromosomes are arranged on the mature spindle. In both nuclei ten chromosomes are visible, and those in the lower nucleus are

already split. A slightly later stage is shown in Pl. XVII, Fig. 18, where in the lower nucleus all the chromosomes have divided and are just on the point of moving apart to form two groups of ten.

After division is complete the four daughter nuclei are again pear-shaped and beaked. The lowest of the four occupies the middle of the ascus and the other three lie rather crowded together in the ascus tip (Text-fig. 25). They remain in these positions until the next division begins.

The third division. At the onset of the final division in the ascus the discrepancy in size of the nuclei is even more marked than before. The lowest nucleus is very much the largest and divides slightly in advance of the other three (Text-fig. 26). The spindle of the lowest nucleus is either parallel to the long axis of the ascus, or rather obliquely placed. This nucleus can be seen in metaphase with all ten chromosomes visible in Pl. XVII, Fig. 19. The other nuclei in this ascus have not been shown as they are all still in prophase and do not give accurate chromosome counts. A later stage is shown in the next figure. Here the lowest nucleus (Pl. XVII, Fig. 20a) is in anaphase, the chromosomes have divided and two groups of eight can be seen. The nucleus and spindle are large, comparable in size with those of the previous division. The other three nuclei are crowded together in the ascus tip (Pl. XVII, Fig. 20b) and the chromosomes have barely divided. It can be seen that the spindles of all these nuclei are considerably smaller than the fourth nucleus already described. The lowest nucleus of an ascus which contained four dividing nuclei is shown at late anaphase in Pl. XVII, Fig. 21. Here the chromosomes have divided and moved apart and a group of ten can be seen at each pole.

As a result of this last division two large nuclei, with well-marked asters, lie in the middle of the ascus (Text-fig. 27), while the other six lie crowded together into the ascus tip. These six nuclei increase hardly at all in size and remain in the same place while spore formation begins round the two large central nuclei (Text-fig. 27).

Spore formation. Two spores only¹ are found in the ascus of *Phyllactinia* and these are formed in relation to the two nuclei occupying the middle of the ascus. The spore membrane appears first in the region of the centrosome. From this point it stretches out through the cytoplasm, following the curve of the astral rays until a portion of cytoplasm containing a nucleus is delimited. At this time the six supernumerary nuclei lie in the ascus tip without any signs of beaks or asters (Text-fig. 28 and Pl. XVII, Fig. 22). The spores increase in size, while the residual cytoplasm becomes scanty and granular, until they very nearly fill the ascus and in doing so push the other six nuclei from their position in the tip round the sides of the uppermost spore into the space between the spores (Pl. XVII, Fig. 23). Here they remain until they

¹ According to Salmond's (1900) monograph, three and even four spores have been reported for *Phyllactinia*. This is a very rare occurrence and has never been seen in the present material.

eventually degenerate and disappear. The spore nuclei do not divide again, so that at maturity the asci each contain two uninucleate spores with only very slightly thickened walls. Germination of the spores has not been seen.

The nuclear history of the ascus is thus a simple one. The two nuclei in the young ascus fuse and after a long period of rest while the ascus trebles its size the fusion nucleus undergoes reduction division. The diploid chromosome number is twenty and the chromosomes are of three different sizes, two pairs being very small. The homotype division is followed by an equational one. Two uninucleate spores are formed and the other six nuclei degenerate.

DISCUSSION

The evidence accumulated from this study of the life-history of *P. corylea* points to the conclusion that the form of this fungus under investigation is apogamous. The evidence in favour of this view may be profitably summarized as follows:

1. Chromosome counts at critical stages in the life-history, from the young sexual branches to the ascogenous hyphae, all give the haploid¹ number; these counts, although not numerous, are individually convincing and present a considerable weight of evidence when viewed as a whole.

2. Nothing has been seen which could be interpreted as the passage of the male nucleus into the oogonium; fusing nuclei, such as are so easily observed in the young ascus, have never been found in the oogonium. This was not the conclusion which was expected when the work was begun.

3. What seems to be a degenerating nucleus can be seen *in situ* in the antheridium after the oogonium has become binucleate.²

4. The degeneration of the male nucleus is progressive from very early in the development of the sexual branches and is well advanced while the oogonium is still uninucleate.

5. The oogonium in this material is constantly uninucleate until it has reached a degree of sheath formation well in advance of that of the fusion stages figured by Harper for his material (cf. Text-figs. 5 and 6 and Harper, Figs. 6 and 7).

6. During the whole of the life-history there is never visible more than the diploid number of chromosomes, and this is only manifested as the ten bivalents at early metaphase (Pl. XVII, Fig. 11) of the meiotic division in the ascus.

The evidence against the apogamous condition is confined solely to the fact that nuclei of two different sizes are present when the oogonium is first seen to be binucleate. This very astonishing difference suggests at once that the

¹ There is no certain way of determining whether the basic chromosome number of this plant is really the *haploid* number; the term is used here as referring to the reduced number found after meiosis.

² This binucleate condition must not be confused with that in the very young oogonial branch which is binucleate before the stalk cell is delimited from the oogonium.

two nuclei might be of different origin and not the result of a division of the oogonial nucleus; that is, that the larger nucleus nearer the base of the cell could be the original nucleus of the oogonium, and that the smaller one could be the male nucleus. On this interpretation the remains in the degenerating antheridium would not embody a nucleus. This explanation, however, underestimates the fact that these remains are so very like a nucleus, with its single persistent nucleolar granule (Text-figs. 6 and 7), and neglects the chromosome counts, both positive pieces of evidence.

The chromosome counts alone, however, do not disallow the hypothesis that a male nucleus has passed into the oogonium. It might be possible, neglecting all the evidence except the chromosome counts and the unequal nuclei, to postulate an association of male and female nuclei in the oogonium, at this stage, and a subsequent fusion of their progeny in the young ascus (i.e. a modification of Claussen's description for *Pyronema confluens*, 1912). This explanation is untenable because the nuclear divisions in the ascogenous hyphae are not conjugate ones, and by no other method could two-thirds¹ of the binucleate ascogenous hyphae cells contain the correct nuclear complement for ascus formation.

A more credible explanation of the discrepancy in size between the two oogonial nuclei is that the original nucleus has divided and the two daughter nuclei have grown at different rates in the manner already described for the nuclei in the ascus. The nucleus at the base of the large cell in this way gets ahead of its fellow after division. These unequal sized nuclei were seen by Harper and commented on by him in his account of *Phyllactinia* (1905, p. 84). They were not considered by him to be the male and female nuclei, fertilization having been described earlier in the development of the perithecium. He also considered them to be the result of a division of the oogonial nucleus, but thought the small nucleus merely an accessory one and unconnected with the main course of the life history. He did not notice the gradual increase in size of the smaller nucleus at successive stages in the further development of the oogonial cell. The final proof of the occurrence of apogamy in *P. corylea*, that of a pair of sexual branches with a nucleus in the antheridium and a dividing nucleus in the oogonium, has not so far been seen, but the weight of evidence presented above seems to be in favour of apogamy as the best explanation of the observed facts.

With regard to the morphology of the perithecium several points are worthy of comment, since the present account differs in three important details from those of earlier workers. These will be discussed in turn.

1. In *Phyllactinia* the perithecium arises always in connexion with two perfectly regular erect branches, which are soon sufficiently different in appearance to be called antheridial and oogonial. These two branches continue their growth by a regular sequence of events. At every stage the young

¹ An average of twenty-four out of a possible thirty-six binucleate cells form asci and more than this begin to grow out in the early stages of ascus formation.

perithecium can be referred back to such a parentage. Furthermore, there is no break in the continuity at any point in the life-history of the central fertile region, and the ascogenous hyphae always arise from cells of the fertile branch. This method of development is substantially the same as that given by de Bary (1887). It differs so fundamentally from the descriptions by Allen (1936) of *E. polygoni* that it is not possible or profitable to compare the two accounts in detail here.

2. In all the earlier accounts of the formation of the row of cells of the fertile region of the young perithecium, the method by which the binucleate oogonium became a row of cells of which only the penultimate was binucleate is nowhere explained. The present description of nuclear division, with the formation of four nuclei and cell-wall formation across the spindles, does provide a sequence of events which leads logically to a row of three cells, of which the middle one must be binucleate (Text-figs. 10, 11, and 12). This arrangement of cells is very reminiscent of crozier formation described for some Ascomycetes (Gwynne-Vaughan and Williamson, 1931, 1932, 1934; Claussen, 1912). The stalk cell of the row may and often does divide again. If it does so, then the well-known and much figured row of cells, with one binucleate one interpolated before the tip cell, results.

3. The ascogenous hyphae at first follow a course similar to that of the oogonial branch. They are multinucleate and non-septate and then, by formation of septa along their length, across the spindles of the last division, a row of cells is produced. By this method the tip cell and the basal cell of each hypha are uninucleate, while those between are binucleate (cf. *Pyronema confluens* Gwynne-Vaughan and Williamson, 1931). In the ascogenous hyphae, as in the oogonial branch, the basal cell of any row may divide again so that more than one uninucleate cell may eventually be found there. The fact that the binucleate cells of the ascogenous hyphae in *P. corylea*, like those of *Geopyxis catinus* (Guilliermond, 1905), grow out to form asci without the intervention of a crozier is interesting. In many Ascomycetes any binucleate cell of the row destined to become an ascus buds out and bends over so that a hook is formed. The two nuclei divide simultaneously and then septa are laid down so that a three-celled crozier results. The middle binucleate cell of these three becomes the ascus. In *Phyllactinia*, as has already been suggested, it is the first few divisions of the young fertile branch (p. 388 and Text-fig. 12) which follow the course as if for crozier formation, and the process is not repeated during the development of the binucleate cells of the ascogenous hyphae into asci. This particular grouping of non-sister nuclei is effected twice in the life-history; in the young fertile branch and in the ascogenous hyphae in *Phyllactinia*; in the ascogenous hyphae and in the crozier in other Ascomycetes such as *Pyronema*. The relationship, if any, of these two life-histories is obscure.

A comparison of the results obtained from the present material with those of Harper in 1905 brings out some very interesting differences. Several of

these, such as the development of the fertile branch and of the ascogenous hyphae, have already been dealt with, but there remain some details especially relevant to the earlier paper. The haploid chromosome number given by Harper is eight and all the chromosomes are more or less equal in size. The number for the plant investigated here is ten and two of the chromosomes are very small. This difference may be a genuine one or it may be that the two small chromosomes were obscured in Harper's material by their larger neighbours. Apogamy with a degenerating nucleus in the antheridium is the rule in this material, whereas Harper describes fertilization for his plant. In one of his figures, however (Fig. 11), there is shown, in a cell described as an empty antheridium, a clear circular area containing a granule commensurate in size with the nucleolus of the antheridial nucleus. This whole structure very much suggests a degenerating nucleus and its presence tempts one to think that perhaps at least some of Harper's material was apogamous like the plant investigated here.

The lateral fusion of chromosomes described by Harper for his fusing nuclei (1905 p. 61,) has not been seen during this investigation. The interpretation of prophase stages is always difficult, and all that can be said for the present material is that during prophase the chromosomes are looped about the nucleolus with occasional free ends visible. The chromosomes are undoubtedly paired in preparation for meiosis at very early metaphase, and at this time the bivalents are variously shaped and very distinct. The question of the fusion of chromosomes during nuclear fusion in the oogonium does not arise in the present material, since the peritheciurn develops apogamously.

SUMMARY

1. The young sexual branches in *Phyllactinia* are erect and coiled about one another and very distinct from the vegetative mycelium. Every peritheciurn begins with the development of such a pair of sexual branches.
2. Antheridial and oogonial cells are cut off before the sheath grows up.
3. The antheridial nucleus degenerates in the antheridium and the oogonium continues its growth without a sexual fusion.
4. The oogonium becomes quadrinucleate by nuclear division, and then, by the formation of septa across the spindles, a three-celled branch is formed of which the middle cell is binucleate.
5. After further nuclear division the ascogenous hyphae grow out from the middle cell of the fertile branch. They are multinucleate and non-septate at first and then septate so that rows of binucleate cells are formed with a uninucleate cell at the top and base of every row.
6. The asci are formed directly from the binucleate cells of the ascogenous hyphae without the intervention of a crozier.
7. The haploid chromosome number of ten is found in all the nuclei in the young peritheciurn and in the ascogenous hyphae.
8. Reduction division follows nuclear fusion. Six nuclei are found in the

ascus at the time of spore formation, but only two uninucleate spores are produced in each ascus.

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EXPLANATION OF PLATES XVI AND XVII.

Illustrating Dr. Barbara Colson's paper on 'The Cytology and Development of *Phyllactinia corylea* Lév.'

PLATE XVI

Fig. 1. Very young sex branches, nucleus in one of the parent hyphae in division; eight chromosomes visible on the metaphase plate. $\times 2,600$.

Fig. 2. Young sex branches after formation of stalk cells; nucleus in the antheridium still unchanged. $\times 2,600$.

Fig. 3. Beginnings of sheath formation from the oogonial branch; antheridial nucleus slightly swollen and showing degenerating chromatin. $\times 2,600$.

Fig. 4. Binucleate oogonium, nuclei slightly different sizes. Antheridium and stalk visible, nucleus in antheridium much degenerated but with nucleolus still clearly present. $\times 2,600$.

Fig. 5. Metaphase of nuclear division in the sheath cell with nucleolus and spindle ends, ten chromosomes visible. $\times 3,400$.

Fig. 6. Same as above, another example. $\times 3,400$.

Fig. 7. Anaphase in a sheath cell, eight rather drawn out chromosomes visible at each pole. The nucleolus is still visible. $\times 3,400$.

Fig. 8. Metaphase in cell at the base of an ascogenous hypha, ten chromosomes visible on the plate. $\times 3,400$.

Fig. 9. Binucleate ascus with the two nuclear areas in contact. $\times 2,600$.

Fig. 10. Fusion of the two nucleoli. $\times 2,600$.

PLATE XVII

Fig. 11. Very early metaphase of first division in the ascus, with two large pairs, six medium-sized pairs, and two very small pairs close together. The nuclear area is still clearly visible and the nucleolus large. $\times 3,400$.

Fig. 12. Metaphase of first division ten pairs of chromosomes; the small granule near the lower edge of the group is the nucleolus. This is the nucleus of the ascus shown in Text-fig. 22. $\times 3,400$.

Fig. 13. Photograph of the same nucleus from text-figure 22; the nucleolus and one pair of chromosomes are out of focus. $\times 1,400$.

Fig. 14. Early anaphase of first division in the ascus; all but two of the pairs have separated: these two are still just joined; nineteen of the twenty chromosomes visible, the nucleolus is on the left-hand side of the spindle. $\times 3,400$.

Fig. 15. Late anaphase of first division; the ascus was cut obliquely and the two groups of chromosomes are at different focal levels with the nucleolus between them; all three levels have been united in the figure; ten chromosomes are visible at each pole. $\times 3,400$.

Fig. 16. Photograph of the lower of the two anaphase groups from Fig. 15. Nine of the ten chromosomes are in focus. $\times 1,400$.

Fig. 17. Metaphase of the second division in the ascus; both nuclei are shown. In the lower nucleus the ten chromosomes are all showing the split; the nucleolus lies on the right. In the upper, the chromosomes are all still unsplit. These are the nuclei in the ascus in Text-fig. 24. $\times 3,400$.

Fig. 18. Early anaphase of the second division. All the chromosomes are split in the lower nucleus and a few halves have already separated; the nucleolus is at the bottom on the right. In the upper nucleus the chromosomes hardly show the split; the nucleolus lies clear of the spindle toward the top of the ascus. $\times 3,400$.

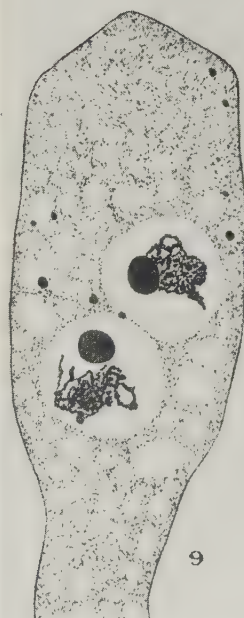
Fig. 19. Metaphase of the third division; ten chromosomes and nucleolus visible. $\times 3,400$.

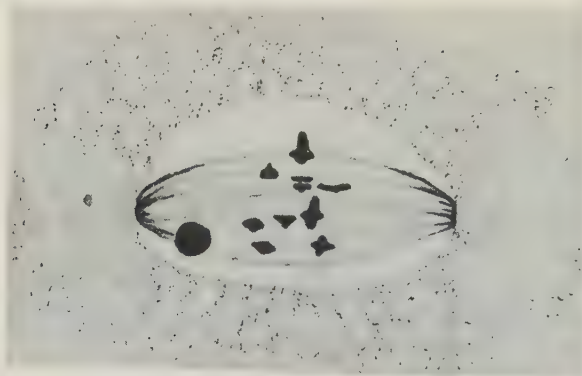
Fig. 20a and b. Two parts of the same ascus with four nuclei in division. (a) The lowest, largest nucleus at early anaphase; two groups of eight and the nucleolus are visible. (b) Three nuclei in the ascus tip all still at metaphase. These nuclei are from the ascus in Text-fig. 26. $\times 3,400$.

Fig. 21. Late anaphase of third division in the ascus, a group of daughter chromosomes at each pole; in the lower group ten are visible. The nucleolus lies to the right. $\times 3,400$.

Fig. 22. Uninucleate spores and six nuclei in the tip of the ascus. $\times 1,300$.

Fig. 23. Uninucleate spores and the other six nuclei degenerating in the space between the spores. $\times 1,300$.

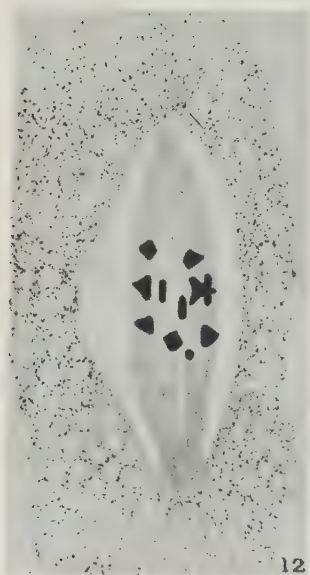




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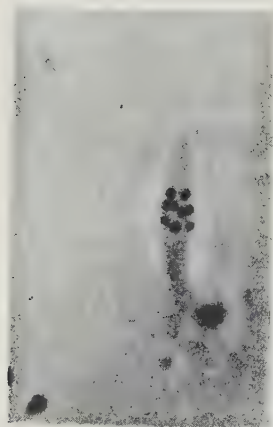
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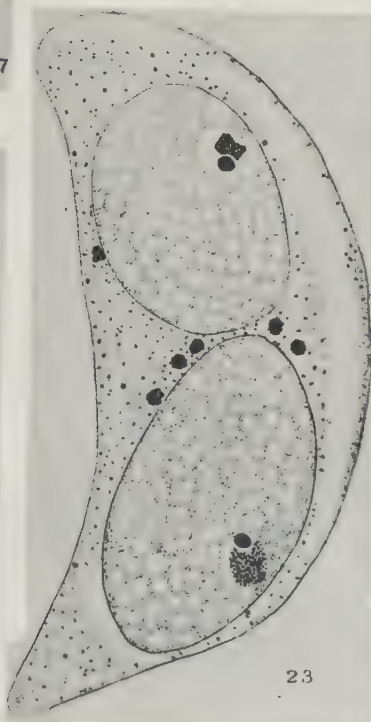
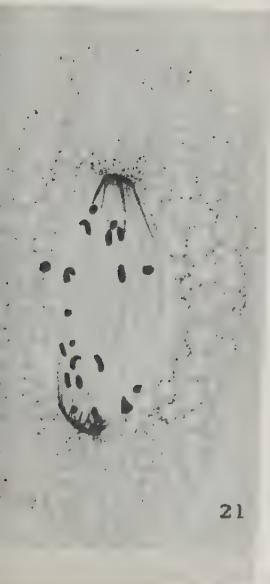
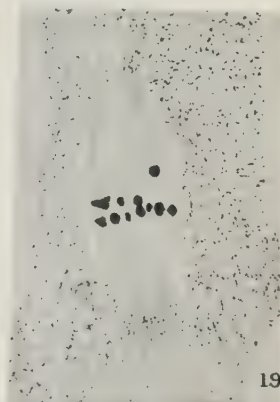
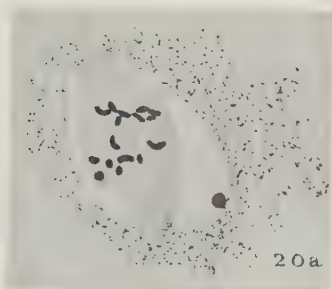
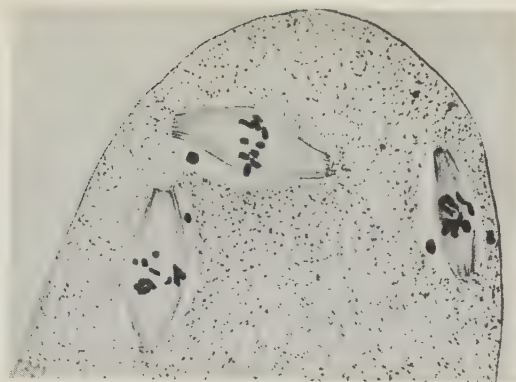
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Physiological Studies in Plant Nutrition

VII. The Role of Fructosans in the Carbohydrate Metabolism of the Barley Plant

Part 2. Seasonal changes in the Carbohydrates, with a Note on the Effect of Nitrogen Deficiency

BY

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With nine Figures in the Text

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I. INTRODUCTION

SINCE the discovery of fructosans by Muntz (1878) a number of records of their occurrence in grasses and cereals has established that these substances are normal constituents of such plants (see Tanret, 1891; Belval, 1924; Colin, 1925; Cugnac, 1931; Kizel and Keretovisch, 1934; Wender, 1919; Norman, 1936). In the cereals wheat, barley, and rye they are reported as occurring in the stems, particularly towards the base, and in the developing ears, and recently they have also been isolated from barley leaves (Archbold and Barter, 1935). It is therefore clear that these carbohydrates may be distributed throughout the plant and should be included in any quantitative survey of the soluble sugars in cereals. Little information is available either as to the amounts of fructosan present in these plants or as to the conditions which govern their appearance. It has already been stated in Part I (Archbold, 1938) of this paper, which deals with the analytical methods adopted for the present work, that

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evidence has been obtained of a relation between fructosan content and nitrogen deficiency, and the data relating to this effect of nitrogen are included in the present paper. A more detailed study of the effect of some other mineral constituents has since been carried out in this laboratory by Russell (1937).

The primary object of the present work was to investigate the seasonal drift of fructosan content both in relation to the other soluble sugars and to the total carbohydrate in leaves, stems, and ears of the barley plant. At the same time an attempt has been made to account for all the assimilated carbon stored in these organs as sugar (including fructosans), fats (ether-soluble fraction), water-insoluble polysaccharides, proteins, and an undefined water-soluble fraction found by difference which will include organic acids, soluble hemicelluloses, &c. For this purpose analyses have been made of the main axes and first tillers of barley plants grown in soil during the two seasons 1935 and 1936. The details of collection and sampling and the analytical methods used are described in detail in Part I (p. 184). The period covered was from the time of emergence of the third leaf until about a month after full emergence of the ear. The final phase of the growth cycle, up to full ripeness of the ear, was to have been studied in 1937, but after the collection of six samples the remainder of the plants was unfortunately devoured by rats.

2. EXPERIMENTAL DATA

Growth curves from the fresh weight data, 1935 and 1936.

The growth curves as found from the fresh weight measurements are shown in Fig. 1, and the fresh weight determinations are included in Table VI. The curves for the complete shoots have the usual sigmoid form and call for no special comment except that heavy rain during the collection of some samples in 1936 made it very difficult to remove all adhering water before weighing, and rather high fresh weight values were obtained on these occasions; such values are marked w in Fig. 1.

In both seasons the maximum leaf weight per shoot was reached just after full expansion of the last leaf, or nine to ten weeks after germination. At this stage there were four to five green leaves remaining on each stem, while at the last collection a month later only one or two leaves remained green. The maximum stem weight was reached about ten days later than that of the leaves. The subsequent fall in the fresh weight of the stems is not clearly shown in the 1936 series, but the six samples collected in 1937 confirm this loss of fresh weight clearly indicated by the 1935 series. In both seasons the ears had not reached their maximum weight when the final samples were taken.

The growth curves for the separate internodes of the stem and the effect of defoliation and removal of the ears on the fresh weight values are shown in Fig. 2 and in Table I.

Defoliation and ear removal were effected after the emergence of the last leaf and before full elongation of the last stem internode. Fig. 2 makes it clear that

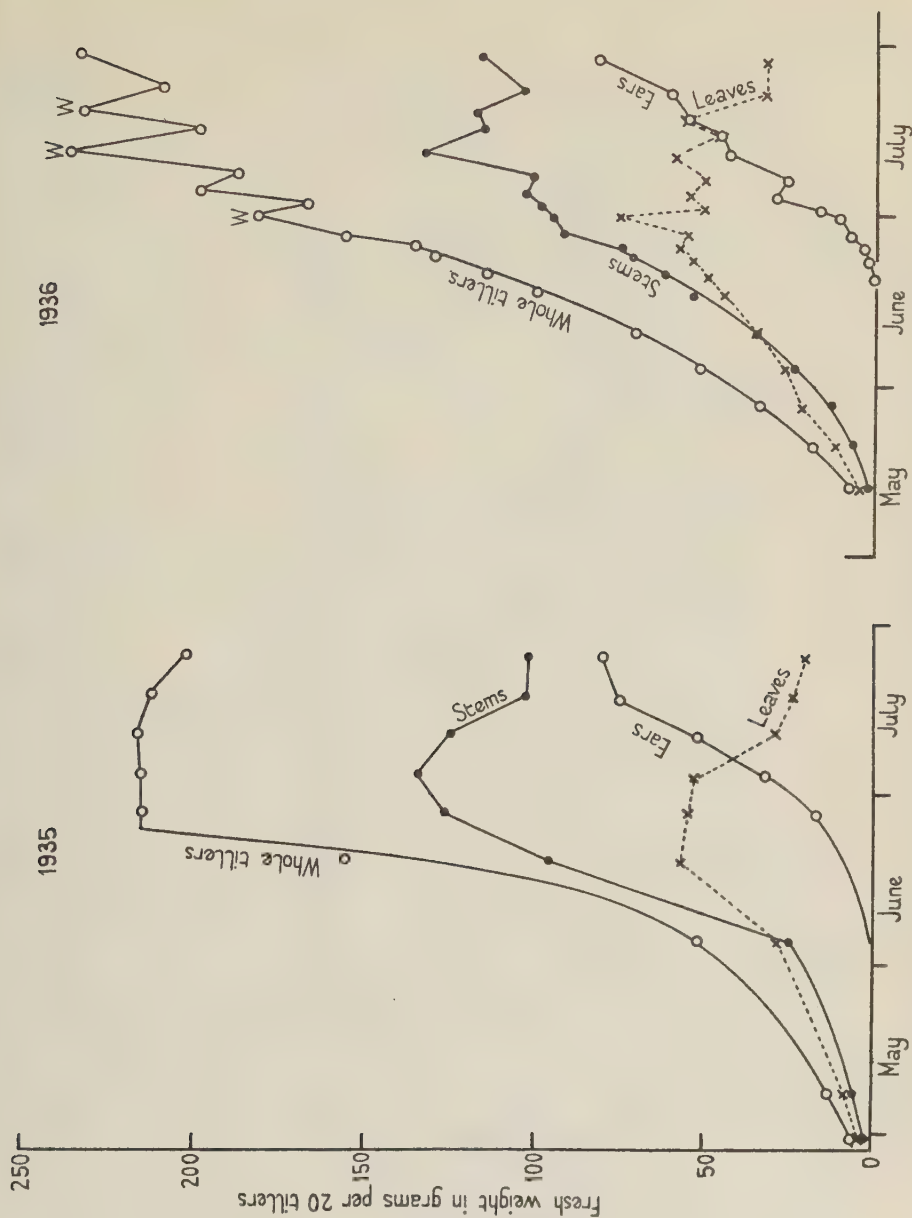


FIG. 1. Fresh weights of twenty whole shoots (main axis and first tiller from each of ten plants) and of the leaves, stems, and ears of barley during growth; 1935 and 1936.

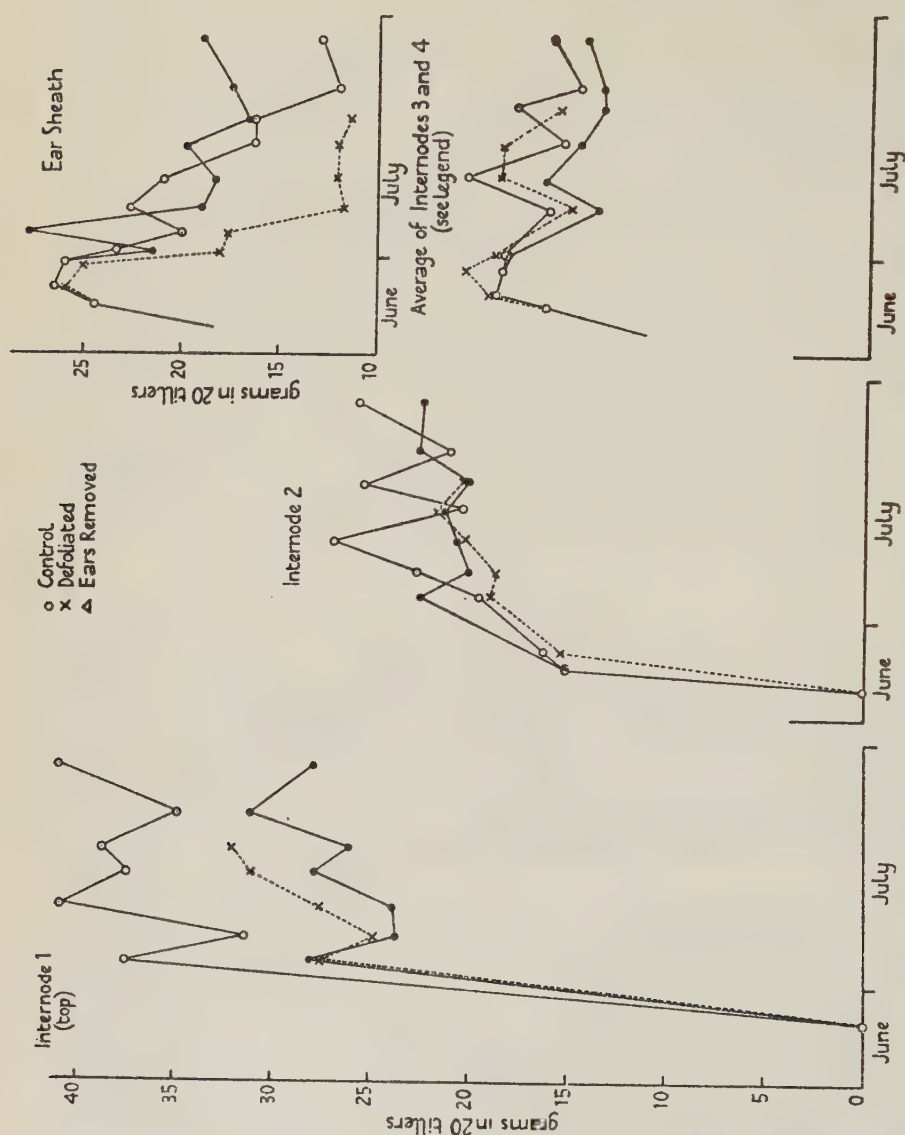


FIG. 2. Fresh weights (grammes per twenty shoots) of ear sheaths and stem internodes of barley after defoliation and ear removal, with controls; 1936. Defoliated 24/6/36; ears removed 29/6/36. (Note: The stems had generally four elongated internodes. The values for 'average of internodes 3 and 4' are half the residual stem weights after removing internodes 1 and 2. In addition to the elongated internodes 3 and 4, a small proportion due to the early unelongated internodes is therefore included.)

TABLE I

Fresh Weight (gm. per 20 Shoots) of the Ears, Peduncles, and Internodes of the Stem of Barley Plants during Growth.
C, controls; F, plants defoliated on 24/6/36; E, ears removed on 29/6/36.

Date.	Ears.		Peduncles.		Ear sheath.			Internode 1 (top).			Internode 2.			Internodes below 2.		
	C.	F.	C.	F.	C.	F.	E.	C.	F.	E.	C.	F.	E.	C.	F.	E.
24/6	3.4	—	—	—	24.5	—	—	—	—	—	15.2	—	—	35.3	—	—
26/6	7.3	7.8	—	—	26.6	26.0	—	0.0	0.0	0.0	16.2	15.5	—	38.2	39.1	—
29/6	10.6	9.0	—	—	26.0	25.1	—	—	—	—	—	—	—	37.6	41.2	—
1/7	17.2	13.7	—	—	23.4	18.1	21.6	—	—	15.0	—	—	23.1	36.8	38.2	36.4
3/7	29.5	11.7	—	—	20.0	17.6	27.6	37.5	27.5	28.6	19.6	19.1	22.6	—	—	—
6/7	25.9	16.2	—	—	22.9	11.8	19.1	32.8	24.8	23.9	22.6	18.5	20.1	32.7	30.2	27.7
10/7	35.9	19.0	7.8	—	20.9	12.1	18.3	40.8	27.4	23.8	26.9	20.7	20.8	40.6	37.8	32.3
14/7	35.2	22.9	10.7	5.7	16.3	12.0	19.8	37.2	31.2	27.9	20.4	21.7	21.4	31.1	36.6	29.4
17/7	43.3	24.8	12.6	6.2	16.3	11.5	16.8	38.8	31.9	26.1	25.4	20.4	20.1	36.6	31.7	27.1
21/7	47.4	—	13.5	—	12.0	—	17.4	34.9	—	31.2	21.1	—	22.6	29.7	—	27.3
27/7	67.0	—	14.8	—	12.9	—	19.2	40.7	—	27.9	24.6	—	22.4	32.1	—	28.7

the elongation of each internode requires only a few days (three to six), and that the maximum fresh weight attained becomes progressively larger from the base upwards. The fresh weight of the two lower internodes fell slowly throughout July, while that of the two upper internodes remained practically constant after the maximum weight was reached. It is, however, apparent from the few samples collected in 1937 that a fall in fresh weight in the upper internodes begins somewhat later than in the lower, so that this stage was not reached before the close of the 1936 experiment.

The effect of defoliation on the fresh weight of the two lower internodes was negligible, but the growth of internodes 1 and 2 (numbered from apex downwards) was somewhat retarded, particularly that of internode 1. Ear removal resulted in a more rapid loss of fresh weight in the lower internodes than in the control plants and also retarded the growth of the upper internodes, the retardation of internode 1 being even greater than after defoliation. It is of some interest that removal of the ear produced an immediate effect in reducing the growth of the stem. The rapid production of new tillers in these plants has already been noted in Part I (p. 185).

In all three series the fresh weight of the ear sheath fell continuously, the loss in the defoliated plants being the greatest. In the defoliated plants ear development continued, but at a slower rate than in the controls, while in the plants from which the ears had been removed the fresh weight of the leaves was on the whole a little lower than that of the controls.

Seasonal drift of sugar content in leaves, stems, and ears.

The seasonal drift of total sugar and of fructosan content is shown in Figs. 3 and 4 and in Table II in terms of the weight of sugar in twenty shoots.

During vegetative development, when the ratio of leaf to stem weight is greater than unity, the sugar content of the leaves steadily increases. The early leaves begin to die off at about the fifth-leaf stage, but the greater size of the later leaves together with a higher sugar concentration results in a continued increase in the amount of sugar per shoot. During stem elongation, when the ratio of leaf to stem weight falls rapidly the amount of sugar in the leaves falls, and in 1935 and 1937 this fall continued, when stem growth was complete, while rapid ear growth was in progress. In 1936 there was a secondary rise in leaf sugar when stem growth ceased. In this series some tillers were removed from the plants after emergence of the last leaf (see Part I, p. 185), and it is suggested that this removal resulted in an accumulation of sugar which would not normally have occurred.

Separate analyses of the two upper and two lower leaves present at any one time were made in 1935 (see Fig. 3). In this series the final fall in sugar content was mainly confined to the lower leaves as they died off, the amount in the upper green leaves remaining constant after full expansion. Whether the fall as senescence begins is due to a failure to replace respiration losses or to a

more rapid rate of translocation of sugar from the dying leaves cannot be ascertained from the present data.

The concentration changes of sugar in the leaves confirm those already noted by Gregory and Baptiste (1936) and Gregory and Sen (1937) in their studies.

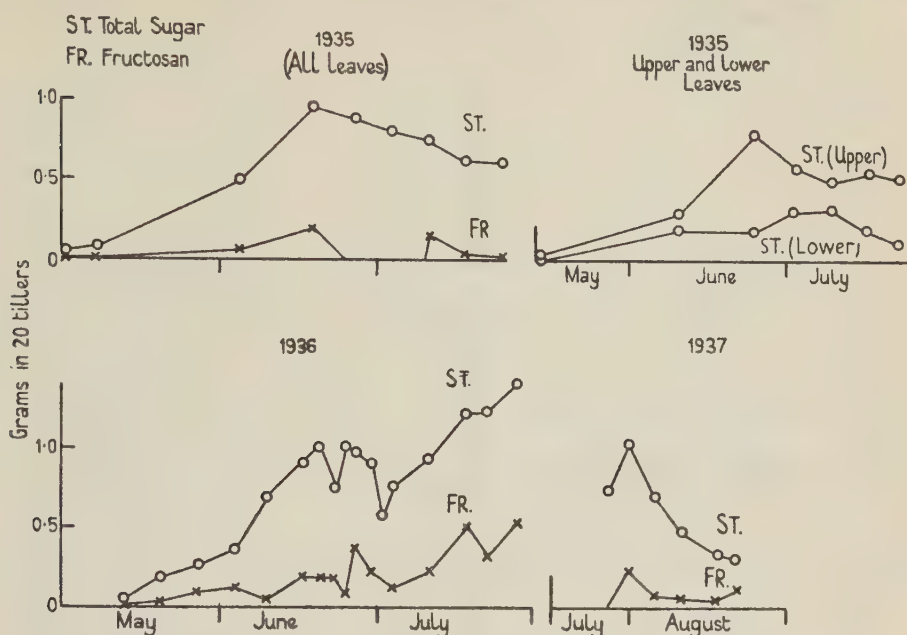


FIG. 3. Total sugar and fructosan contents (grammes in twenty shoots) of barley leaves during growth; 1935, 1936, and 1937.

of single leaves on the main axis. Sugar concentration falls until the fourth leaf emerges, covering the period of active tillering, and subsequently there is a steady rise until rapid stem elongation begins (ninth leaf), after which there is a secondary fall. During ear growth the concentration again rises, predominantly owing to a relatively large fall in water content, not to a real increase in the total amount of sugar present (see p. 404). No account has been taken here of variations produced by diurnal changes or by climatic conditions. The general similarity of the seasonal drift in the two years and the agreement with the data of Gregory suggest that by sampling at a fixed time of day fluctuations due to these factors fail to mask the general drift with time.

Up to the emergence of the seventh or eighth leaf the aerial parts of the plant other than leaf blades consist principally of the leaf sheaths. These have initially a very low sugar content (see Fig. 4), and at the fourth-leaf stage contain in aggregate only 30 per cent. of the total sugar present; at the eighth-leaf stage the proportion has risen to 50 per cent., and when the stems are fully elongated they contain 70 per cent. of the total sugar; at this stage only

15 per cent. is found in the leaves, the remaining 15 per cent. being in the ears. Sugar thus increases in the stems relatively faster than in the leaves, and continues to rise when leaf sugar is falling. In 1935 and 1937 a maximum was reached about ten days later than in the leaves, but as the ears developed

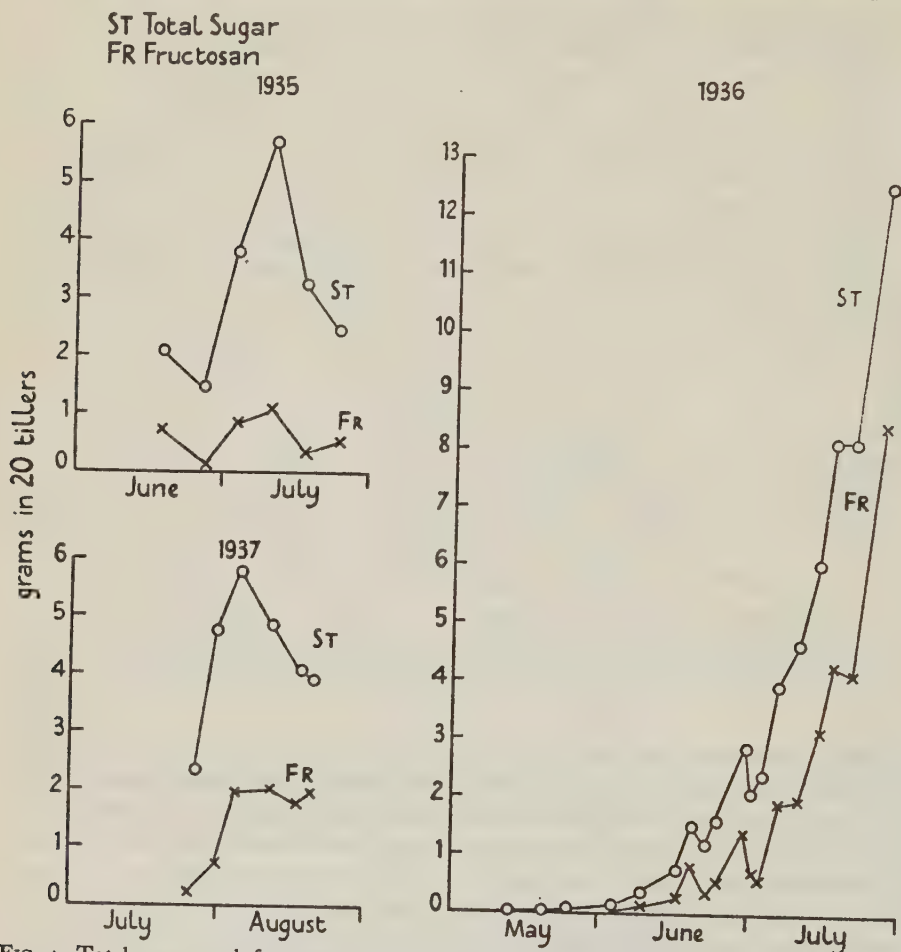


FIG. 4. Total sugar and fructosan contents (grammes in twenty shoots) of barley stems during growth; 1935, 1936, and 1937.

the sugar in the stem fell. In 1936 the sugar content of the stems continued to rise until the last collection, possibly as a result of removal of tillers as already noted.

Rapid ear development began about four days after the beginning of stem elongation, accompanied by sugar accumulation in the ears. These experiments were not carried on sufficiently long enough to obtain evidence of a fall in sugar as the ear ripens, but it is well known that sugar ultimately decreases in amount as full ripeness is reached.

TABLE II

Sugar Content of the Leaves, Stems, and Ears of Barley during Growth. Total Sugar (after Hydrolysis with N/5 Acid) S_T , Reducing Sugar S_R , Fructosan F_r , Sucrose S_s , and $(S_T - (S_R + F_r + S_s)), f$.

All results stated as gm. per 20 shoots (averages of duplicate values)

Date.	Leaves.					Stems.					Ears (with peduncles).				
	S_T	S_R	F_r	S_s	f	S_T	S_R	F_r	S_s	f	S_T	S_R	F_r	S_s	f
30/4	0.048	0.003	0.008	0.039	—	2.02	0.70	0.71	0.23	0.38	0.69	0.29	0.22	0.07	0.11
7/5	0.062	0.009	0.005	0.056	—	1.43	0.95	0.18	0.19	0.11	1.05	0.63	0.07	0.20	0.15
4/6	0.49	0.08	0.06	0.33	0.03	3.78	1.08	0.85	0.99	—	1.05	—	—	—	—
18/6	0.96	0.13	0.19	0.48	0.17	5.69	3.32	1.04	1.32	—	—	—	—	—	—
26/6	0.89	0.32	0.00	0.57	0.00	3.20	1.87	0.31	0.65	0.37	1.80	0.54	0.51	0.59	0.16
3/7	0.80	0.32	0.01	0.39	0.08	2.42	1.28	0.51	0.54	0.09	1.10	0.39	0.18	0.62	0.00
10/7	0.75	0.25	0.15	0.41	0.00	—	—	—	—	—	—	—	—	—	—
17/7	0.62	0.19	0.03	0.33	0.07	—	—	—	—	—	—	—	—	—	—
24/7	0.61	0.21	0.02	0.34	0.04	—	—	—	—	—	—	—	—	—	—
1935															
12/5	0.041	0.007	0.013	0.023	—	0.012	0.003	0.004	0.003	0.02	0.03	0.04	0.12	0.03	0.00
19/5	0.185	0.026	0.035	0.123	—	0.044	0.018	0.020	0.005	—	0.44	0.10	0.24	0.06	0.05
26/5	0.264	0.045	0.093	0.127	—	0.071	0.026	0.032	0.013	—	0.58	0.17	0.35	0.04	0.03
3/6	0.362	0.073	0.119	0.170	—	0.144	0.062	0.055	0.026	—	0.67	0.30	0.27	0.05	0.06
9/6	0.682	0.082	0.053	0.547	—	0.351	0.139	0.128	0.085	—	1.06	0.55	0.31	0.14	0.07
16/6	0.896	0.135	0.194	0.563	—	0.740	0.319	0.297	0.119	—	0.91	0.45	0.34	0.12	0.10
19/6	1.00	0.19	0.10	0.62	—	1.50	0.49	0.79	0.21	—	1.21	0.59	0.34	0.30	0.03
22/6	0.75	0.16	0.18	0.39	—	1.21	0.66	0.35	0.19	—	0.03	—	—	—	—
24/6	1.01	0.23	0.09	0.69	—	1.53	0.60	0.52	0.38	0.04	0.20	0.04	0.12	0.03	0.00
26/6	0.97	0.28	0.37	0.33	—	—	—	—	—	—	0.44	0.10	0.24	0.06	0.05
20/6	0.90	0.28	0.23	0.43	—	2.83	0.70	1.44	0.62	0.07	0.58	0.17	0.35	0.04	0.03
1/7	0.57	0.28	0.08	0.21	—	2.03	0.73	0.75	0.51	0.05	0.67	0.30	0.27	0.05	0.06
3/7	0.76	0.12	0.11	0.53	—	2.33	0.92	0.61	0.78	0.03	1.06	0.55	0.31	0.14	0.07
6/7	—	—	—	—	—	3.91	0.84	1.91	1.16	0.01	0.91	0.45	0.34	0.12	0.10
10/7	0.93	0.17	0.23	0.52	—	4.54	0.77	1.97	1.64	0.16	1.21	0.59	0.34	0.30	0.03
14/7	—	—	—	—	—	6.00	0.74	3.13	1.47	0.67	1.84	0.50	0.85	0.50	0.29
17/7	1.22	0.18	0.51	0.54	—	8.07	0.47	4.26	1.72	1.68	2.25	0.43	1.14	0.74	—
21/7	1.23	0.14	0.32	0.78	—	8.07	0.41	4.13	2.44	1.09	2.71	0.47	1.07	1.06	0.10
27/7	1.41	0.22	0.54	0.65	—	12.19	0.50	8.42	2.85	0.43	4.62	0.38	2.17	1.66	0.41

The amount of fructosan in the leaves was always small, and frequently only traces were found (see Fig. 3). In both seasons there was a small increase during leaf development, but in 1935 fructosan subsequently almost disappeared, while in 1936 the fructosan content rose when the secondary rise in sugar occurred. In this instance conditions (i.e. the removal of some tillers) producing an abnormal storage of sugar resulted in an increase both in the amount of fructosan and in the proportion relative to total sugar. In the stems the amount of fructosan was slightly higher than in the leaves during leaf development, and stem elongation was accompanied by a marked rise. In 1935, at the time of maximum sugar content in the stems, 20 per cent. consisted of fructosan, while in 1936 the proportion rose to 50 per cent., and a similar proportion was found in the ears. These observations confirm the results of previous workers in showing that under normal conditions of growth there is a drift of increasing fructosan in the plant with age and an accumulation in the stems and ears. In addition, however, they show that small amounts normally occur in the leaves, but these undergo no important changes: fructosan does not accumulate at any stage in the leaves unless special environmental conditions are operative, tending to abnormal sugar accumulation. There is also some indication that the stored fructosan is finally translocated from the stems and undergoes conversion to other substances in the ears.

The amounts of reducing sugar and of sucrose present during growth are also shown in Table II; since the amounts of free fructose and free glucose were throughout approximately equal, these sugars are not separately recorded. Where the amount of apparent sucrose determined by invertase action was less than the amount determined as the difference between the total increase on acid hydrolysis and the fructosan estimate, the lower value is given in Table II (see Part I, p. 196). In these cases there is an excess of sugar produced by acid hydrolysis whose origin cannot be defined but which probably arises from a fructosan containing more 'glucose' than the one already isolated (see Part I, p. 197). This fraction is given in columns *f* in Table II. It must be emphasized that the sucrose estimates under these conditions are not very accurate, and are certainly too high, while those of fructosan are low.

In the 1936 series the amount of sucrose in the leaves remained throughout between 50 and 60 per cent. of the total sugar, with a tendency for the proportion to fall with increasing age. In the early leaves (up to the sixth) the remaining sugar consisted of 30 per cent. fructosan and 20 per cent. reducing sugar. The proportion of reducing sugar rose to 35 per cent. as the last leaf expanded, after which the amount remained constant, and in consequence the proportion fell to 15 per cent. during the secondary rise in total sugar. Conversely the proportion of fructosan fell at first to 15 per cent. and during stem elongation and ear development rose to 35 per cent. It will be seen in Table II that the absolute amounts of fructosan and reducing sugar in the early leaves are both very small, and the changes in these sugars during leaf development are

relatively unimportant in relation to the total carbohydrate changes. In 1935, when additional nitrate was added to the plot, only traces of fructosan were found in the leaves, and the proportion of sucrose was correspondingly higher. The barley leaf, in common with other leaves, thus contains sucrose as the principal sugar, and changes in other sugars are of little significance. In the very early leaves (up to the third) it is doubtful if the presence of reducing sugar can be satisfactorily demonstrated at all since the amount is so small and interference with the estimation large (see Part I, p. 194).

In the stems reducing sugar and fructosan were also present in equal proportions during leaf development but to the extent of 40 per cent. each of the total sugar, leaving only 20 per cent. of sucrose. When stem elongation began there was no further increase in reducing sugar and the proportion fell rapidly until finally only 5 per cent. of the total was in this form, while at the same time fructosan increased to 50 per cent. of the total. In 1935, with the high nitrogen level, the amount of sugar stored in the stems was far less than in 1936, and at the time of the sugar maximum reducing sugars constituted 50 per cent. of the total and fructosan 20 per cent. In the stems therefore there is relatively much less sucrose than in the leaves, and the remaining sugar may consist either mainly of fructosan or of reducing sugar according to the conditions of growth.

In the ears initially 60 per cent. of the sugar was present as fructosan and about 20 per cent. each of sucrose and reducing sugars. During stem elongation reducing sugars accumulated, while the other sugars showed no material change, but when stem growth ceased there ensued a large increase in sucrose and fructosan with no further increase in reducing sugars. At this stage also, both in the stems and the ears, there is evidence of a relatively large increase in the *f* fraction (see Table II), and indeed, formation of any measurable quantity of this fraction is confined to this stage.

It is clear from the data presented above that there is a general accumulation of soluble sugars in the aerial parts of the plant until stem elongation is complete. During ear development the total amount begins to decline slowly and there is a considerable transference from stems to ears. This decline may be delayed, as in the 1936 experiment, by special external conditions limiting sugar utilization. The ratio of total sugar in the leaves to that in the stems is at first high and remains greater than unity until the eighth leaf is emerging. As the stem elongates the ratio gradually falls until finally 70 per cent. of the sugar is present in the stems. Up to the eighth-leaf stage sucrose is present to a greater extent than other sugars, but as sugar storage in the stems proceeds the proportion falls until reducing sugar, fructosan, and sucrose are each present to approximately equal extents. Finally, when stem growth ceases and excess of fructosan over sucrose is stored the percentage of sucrose falls to 25 per cent. and that of fructosan rises to 50 per cent. This general drift is in accordance with the view that sucrose is exported from the leaves and at some stage in translocation is hydrolysed to reducing sugars. If these sugars

are not at once used for purposes of synthesis they undergo slow conversion to fructosan. Moreover, it appears that fructosan formation is especially favoured by conditions which delay the utilization in further growth of temporary stores of sugar, as, for example, by tiller removal. The distribution of reducing sugars, sucrose, and fructosan in leaves, stems, and ears in terms of percentage of sugar present is shown in Fig. 5.

Sugar content of successive internodes of the stem.

In 1936, after the emergence of the last leaf, ears, peduncles, ear sheaths, and the two uppermost internodes of the stem were separately analysed and the lower internodes taken together as a group. The results are shown in Table III and in Figs. 6 and 7. The lower internodes consisted of two (occasionally three) elongated internodes (3 and 4) and a small proportion representing the remaining internodes. In Fig. 6 the values given for average of 'internodes 3 and 4' represent half those obtained for the whole lower group. Throughout the observed period there was a continuous increase in sugar concentration in each internode, including the peduncle. Concentration in the ear sheath fluctuated but showed no definite trend, and in the ear there was a tendency for concentration to fall. As the last two internodes developed a gradient of increasing concentration was established from apex to base, but subsequently storage of sugar was relatively greater in the upper internodes (1 and 2) and the maximum concentration shifted to internode 2. At the last collection the concentrations in internodes 2, 3, and 4 were above 10 per cent. of the fresh weight, or 30 per cent. of the dry weight. A similar trend of sugar was found by Went (1898) in the internodes of the sugar cane, but in this case the falling concentration of reducing sugar from apex to base was accompanied by a rising concentration of sucrose alone. The concentrations of total sugar reached at the base of the barley stem were actually as high as those recorded by Went for the sugar cane.

If the total amount of sugar per internode be considered (Table III) it is found that during rapid stem elongation the amount of sugar in each internode is approximately equal, but as the upper internodes reach their maximum size the amount of sugar present falls from apex to base of the stem in contrast with the concentration gradient. It has already been noted that the upper internodes are large in comparison with the rather woody ones towards the base, and it is evident that the rather high concentration developed in the lower internodes is due not only to sugar storage but to water loss, as is shown by the falling fresh weights in Fig. 2. Thus on July 10, 50 per cent. of the sugar was stored in internodes 1 and 2, although concentration was then highest at the base, and at the end of July, when the maximum concentration was in internode 2, 63 per cent. of the sugar was in the two uppermost internodes.

The gradient of reducing sugar concentration was opposite to that of total sugar, while that of fructosan and of sucrose was similar. Owing to the

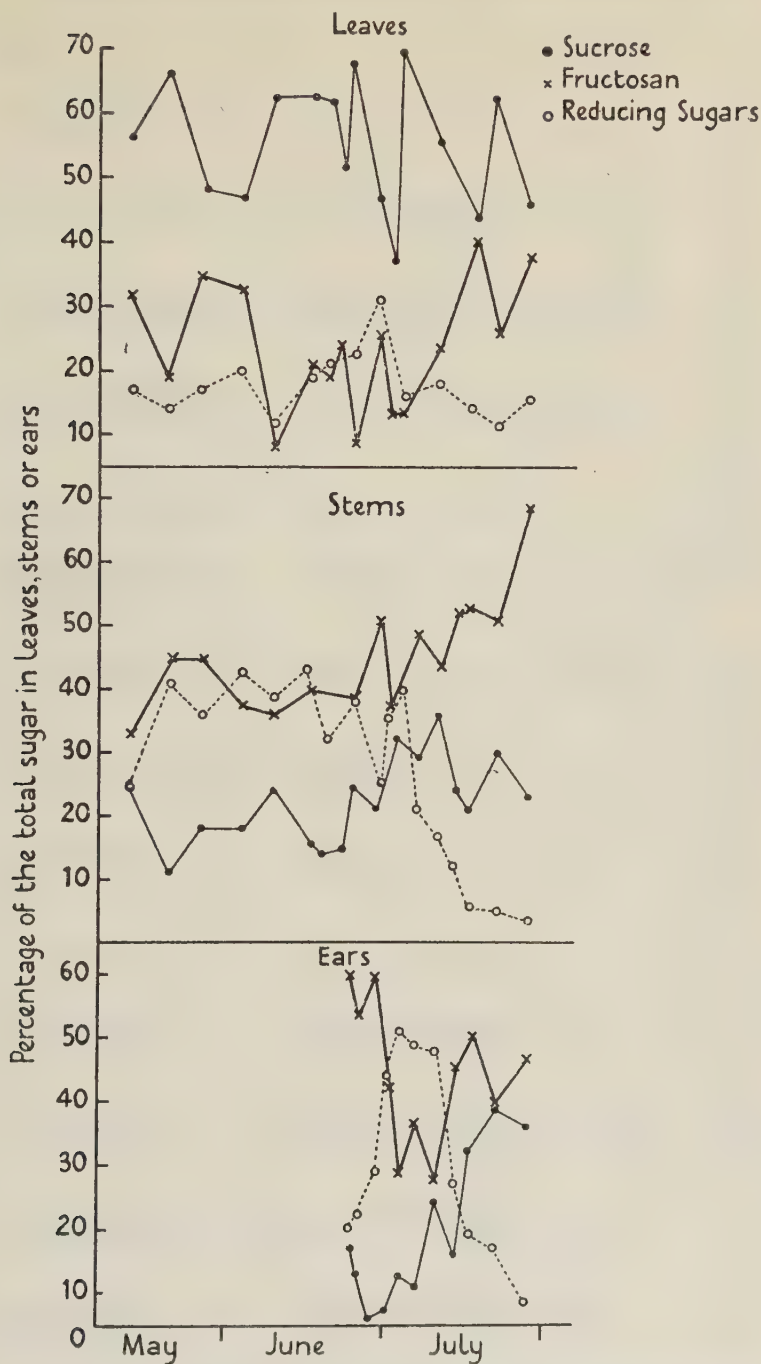


FIG. 5. Fructosan, sucrose, and reducing sugar contents (as percentage of the total sugar) in barley leaves, stems, and ears during growth; 1936.

TABLE III

Sugar Content of Different Parts of the Main Axis of the Barley Plant. Total Sugar (after Hydrolysis with N/5 Acid) S_T , Reducing Sugar S_R , Fructosan F_r , Sucrose S_s , and $(S_T - (S_R + S_s + F_r))f$.

All results stated as gm. per 20 shoots (averages of duplicate values)

[illegible]

<i>Defoliated</i>									
Ears (with peduncles)					Ear sheaths		Internode 1		
26/6	0.28	0.11	0.08	0.09	0.33	0.21	0.05	0.07	—
29/6	0.22	0.14	0.02	0.06	0.15	0.12	0.00	0.03	—
1/7	0.28	0.23	0.02	0.02	0.17	0.09	0.00	0.09	—
3/7	0.28	0.21	0.03	0.05	0.19	0.08	0.00	0.11	0.03
6/7	0.34	0.24	0.04	0.06	0.12	0.06	0.01	0.05	0.06
10/7	—	—	—	—	0.14	0.06	0.00	0.11	0.14
14/7	0.88	0.40	0.17	0.38	0.19	0.03	0.01	0.14	0.10
17/7	0.46	0.16	0.15	0.15	0.07	0.02	0.01	0.05	0.09
All internodes below 2									
26/6	0.07	0.05	—	—	0.31	0.22	—	—	—
29/6	—	—	—	—	0.07	0.03	—	—	—
1/7	—	—	—	—	0.06	0.02	—	—	—
3/7	0.03	0.01	—	—	—	—	—	—	—
6/7	0.05	0.01	—	—	0.08	0.01	—	—	—
10/7	0.19	0.02	—	—	0.32	0.08	—	—	—
14/7	0.25	0.05	—	—	0.39	0.07	—	—	—
17/7	0.22	0.07	—	—	0.32	0.08	—	—	—
<i>Ears removed</i>									
Leaves					Ear sheaths		Internode 1		
1/7	1.54	0.37	0.09	1.08	0.80	0.27	0.35	0.17	0.16
3/7	1.08	0.12	0.34	0.56	0.94	0.27	0.52	0.16	0.14
6/7	—	—	—	—	0.99	0.18	0.52	0.29	0.07
10/7	1.73	0.22	0.98	0.53	1.04	0.15	0.65	0.24	0.08
14/7	—	—	—	—	0.83	0.12	0.41	0.29	0.12
17/7	1.43	0.25	0.51	0.67	0.67	0.12	0.34	0.21	0.35
21/7	1.78	0.17	0.65	0.97	0.77	0.10	0.40	0.28	0.71
27/7	1.60	0.21	0.61	0.78	0.86	0.13	0.41	0.32	0.10
All internodes below 2									
1/7	0.71	0.24	0.18	0.30	1.00	0.24	0.39	0.37	0.83
3/7	0.89	0.17	0.27	0.45	—	—	—	—	1.51
6/7	1.52	0.10	0.75	0.67	1.89	0.09	0.87	0.65	2.13
10/7	2.27	0.06	1.48	0.70	2.99	0.08	1.47	0.93	3.21
14/7	2.62	0.05	1.65	0.65	2.79	0.06	1.84	0.67	2.25
17/7	1.80	0.07	1.21	0.43	2.10	0.07	1.20	0.49	3.68
21/7	2.66	0.09	1.93	0.51	2.30	0.06	1.67	0.60	3.98
27/7	2.85	0.09	2.22	0.51	3.08	0.08	2.13	0.62	0.16
All internodes below 2									
1/7	0.71	0.24	0.18	0.30	1.00	0.24	0.39	0.37	0.05
3/7	0.89	0.17	0.27	0.45	—	—	—	—	0.72
6/7	1.52	0.10	0.75	0.67	1.89	0.09	0.87	0.65	0.54
10/7	2.27	0.06	1.48	0.70	2.99	0.08	1.47	0.93	1.07
14/7	2.62	0.05	1.65	0.65	2.79	0.06	1.84	0.67	1.99
17/7	1.80	0.07	1.21	0.43	2.10	0.07	1.20	0.49	1.35
21/7	2.66	0.09	1.93	0.51	2.30	0.06	1.67	0.60	2.64
27/7	2.85	0.09	2.22	0.51	3.08	0.08	2.13	0.62	2.71

diminishing size of the internodes from apex to base, the amounts of reducing sugar will, of course, also be least at the base. The amount of fructosan rises in the lower during growth of the upper internodes, which in conjunction with

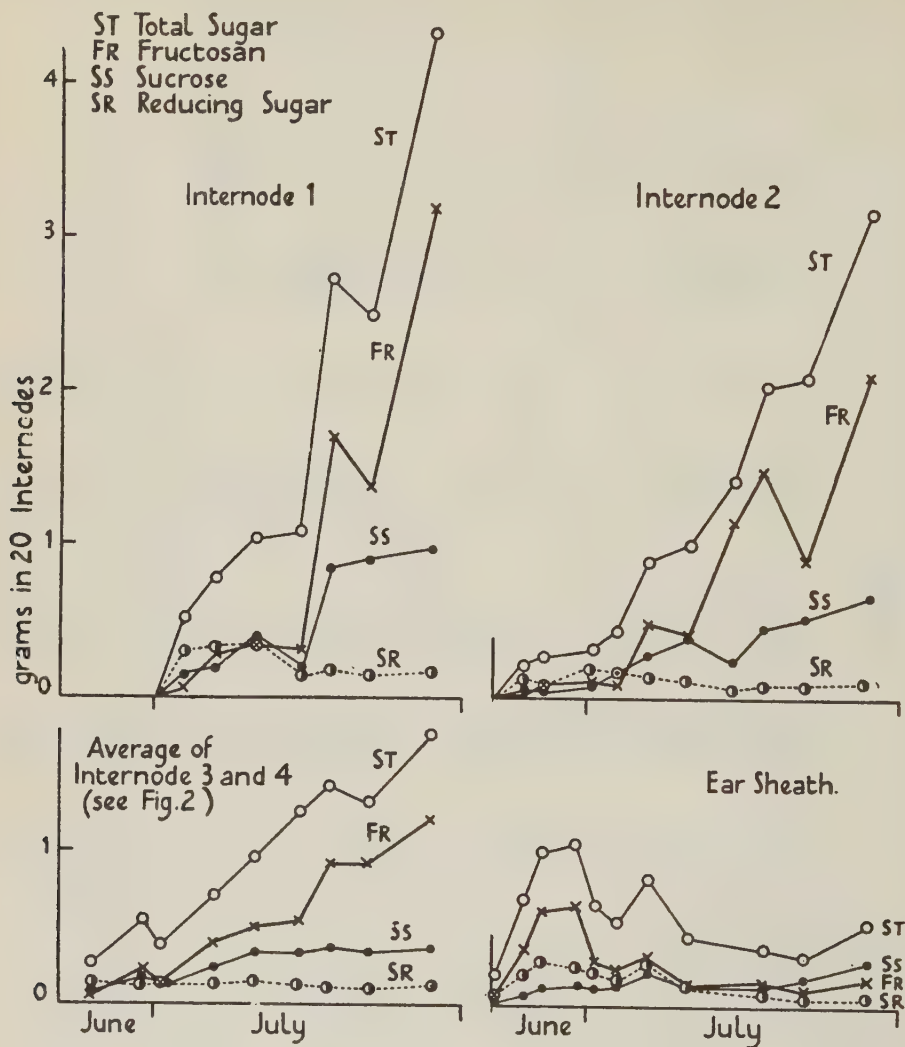


FIG. 6. Total sugar, fructosan, sucrose, and reducing sugar contents (grammes in twenty shoots) of ear sheaths and internodes of the stems of barley during growth; 1936. (See Note to Fig. 2.)

the falling reducing sugar content is in agreement with the view already expressed that reducing sugar is converted to fructosan. When all the internodes are fully elongated increase in reducing sugar and in sucrose ceases, and the whole increase in sugar is due to fructosan, so that finally 70 per cent. of the sugar at the base is in this form. In internode 2 the same drift in sugars

occurs but a little later, and again 70 per cent. of the sugars is stored as fructosan. In internode 1 the amount of fructosan reached 56 per cent. of the total sugar, there being a higher proportion of sucrose in this case, and similar proportions are found in the peduncle. In the ear sheath relatively large amounts

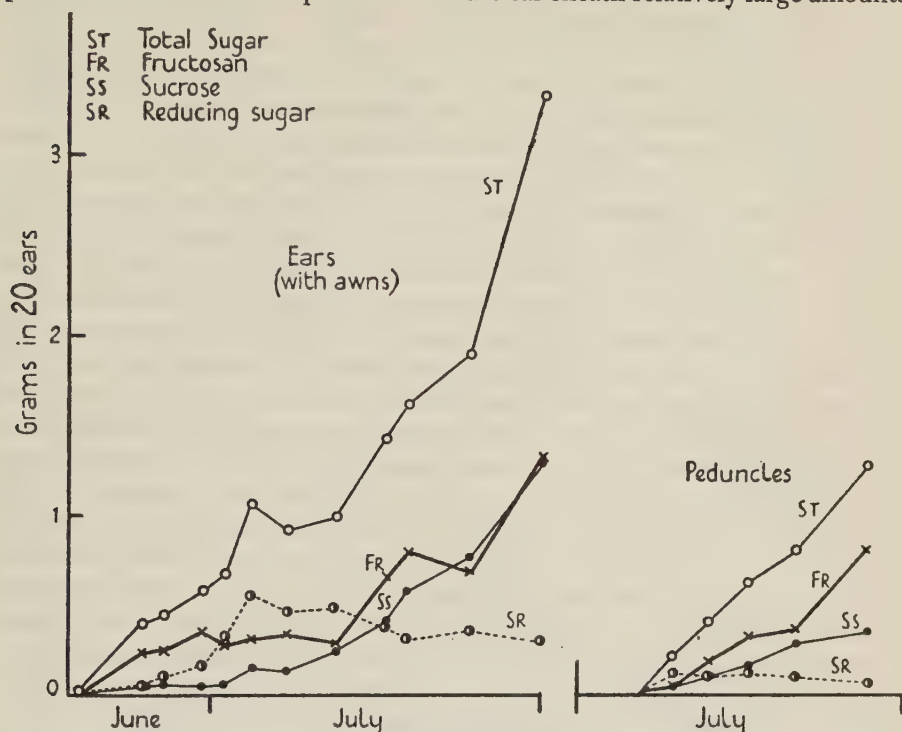


FIG. 7. Total sugar, fructosan, sucrose, and reducing sugar contents (grammes in twenty shoots) of barley ears (with awns) and peduncles during growth; 1936.

of fructosan are present at first, but after its growth has ceased there is a continuous loss of sugar, mainly fructosan and reducing sugar, little change being observed in sucrose. During growth of the sheath, therefore, sugar is stored to some extent, and later presumably this sugar forms a source of supply for the ear during the early stages of development. This loss of fructosan from the ear sheath, together with the high proportion found in the peduncle, makes it almost certain that stored fructosan is readily utilized again at the growing-points, although in this series of analyses it was not possible to demonstrate loss of fructosan from the internodes of the stem itself as the experiments were not sufficiently prolonged (see Figs. 6 and 7).

Effect of defoliation and ear removal on the sugar content.

The sugar content of samples after defoliation and ear removal is shown in Table III. In the defoliated plants all the soluble sugar including fructosan disappeared from the stems within a week. When the restricted growth of the

upper internodes ceased there followed a small increase in sucrose, but no evidence was obtained of any formation of fructosan. In the ears accumulation of sugars was reduced almost to zero, and no fructosan appeared. The translocated sugar was therefore insufficient to supply the demands of protein and polysaccharide synthesis, resulting in the absence of residual free sugar and in restricted growth as compared with the controls. Ear removal conversely resulted at first in a general increase in sugar content over that of the controls. Subsequently, when the growth in the mutilated shoots ceased the sugar content of the fully grown control plants reached the same high level. In both leaves and stems the increase was due to fructosan and sucrose, there being no change in reducing sugars. The effect of ear removal on the fructosan and sucrose contents of the separate internodes of the stem is shown in Fig. 8. It will be seen that in both treated and control plants approximately equal amounts of sucrose and fructosan are produced in each series while the internodes are still extending. When extension is complete there follows at once an accumulation of fructosan and only little further increase of sucrose. The retardation of growth following ear removal thus accelerates the process of fructosan storage, and as the upper internodes are considerably smaller than those of the controls (see Fig. 2) higher concentrations (up to 14 per cent. fresh weight) are reached in the mutilated plants. In the lower internodes, which were already fully extended when the ears were removed, the same effect is seen but to a less marked degree, the bulk of the fructosan resulting from ear removal appearing in the two uppermost internodes. The rapid storage of fructosan in fully elongated internodes is again accompanied by an increase in the undefined *f* fraction. Appearance of the suggested second fructosan is thus related to the cessation of sucrose storage, but without a more certain estimate of sucrose the quantitative relationships are not very clear.

Effect of nitrogen deficiency on the fructosan content.

It is well known that in general nitrogen deficiency results in an increase in the concentration of soluble sugars, and this effect has been clearly shown for barley leaves by Gregory and Baptiste (1936) and Gregory and Sen (1937). Their analyses were made on solutions prepared from alcoholic extractions and would therefore include only a small part of any fructosan present. Evidence has now been obtained that an important effect of this deficiency is an increase in fructosan content as well as in the other soluble sugars, and that in leaves normally containing only traces of fructosan nitrogen deficiency may result in an accumulation of considerable amounts of this sugar. This relationship was first suggested by the remarkably low fructosan content of plants grown in soil which had received a large dressing of nitrate, and has now been confirmed by two series of analyses, one on plants grown in soil, and one with sand cultures. The details of collection and sampling of these plants are described in Part I, p. 184, and the results obtained are presented below in Tables IV and V. These figures confirm the observations of Gregory

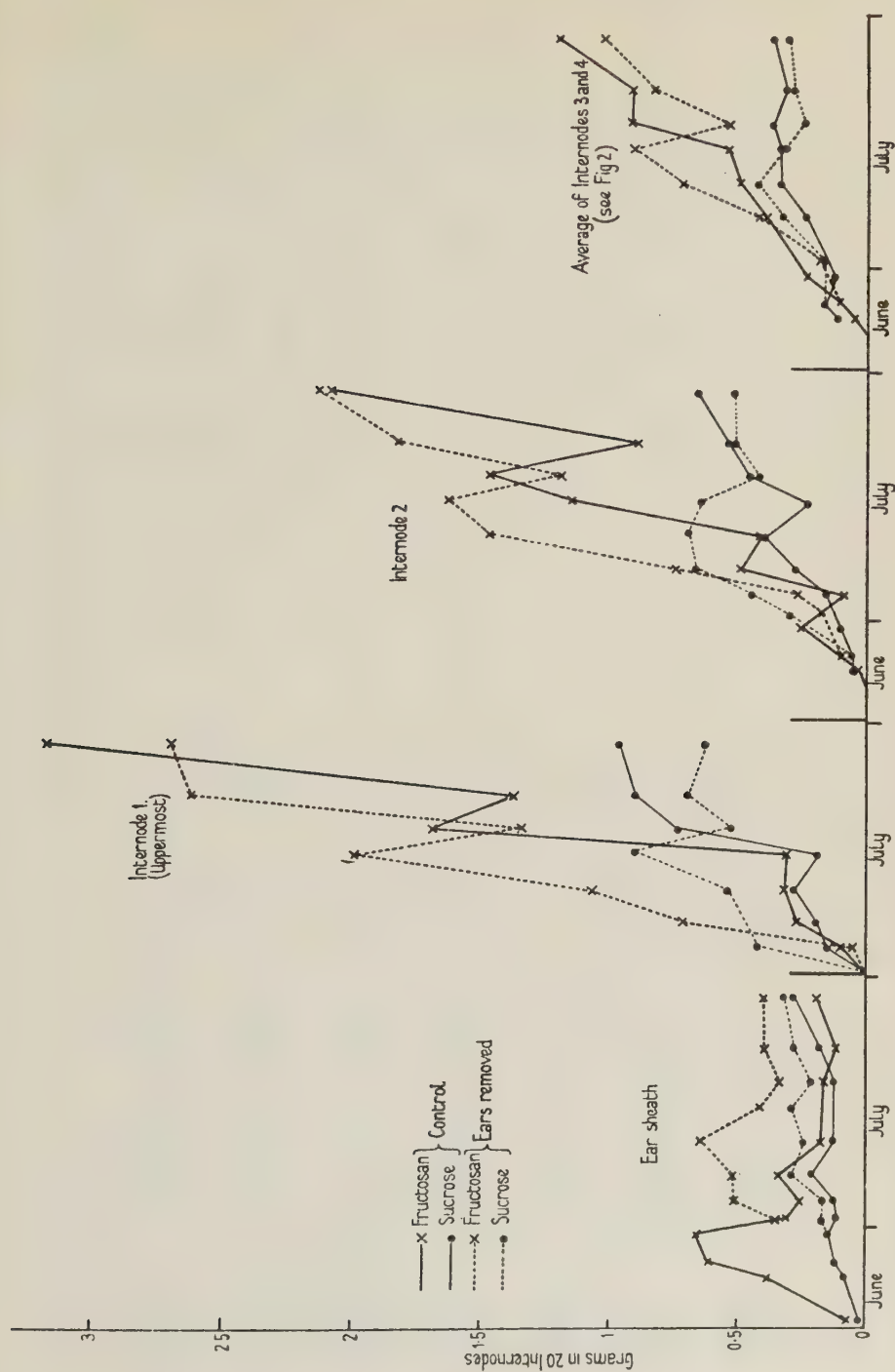


FIG. 8. The effect of ear removal on the fructosan and sucrose contents of ear sheaths and internodes of the stem in barley. (See Note to Fig. 2.)

TABLE IV

Reducing Sugar, Fructosan, and Sucrose Contents of Barley Leaves and Stems from Plants grown in Soil and receiving Dressings of N, P and K, and NPK.

Results are means of duplicate analyses and expressed as percentage of the fresh weight.

Date of collection	Leaves.					Stems.				
	control.	K.	P.	N.	NPK.	control.	K.	P.	N.	NPK.
<i>Reducing sugars</i>										
6/6/35	0.69	0.48	0.48	0.90	0.71	0.61	0.58	0.60	1.02	0.87
25/6/35	1.24	1.00	1.20	0.56	0.89	1.32	0.83	1.35	1.78	1.34
9/7/35	0.91	1.00	0.85	1.16	1.06	1.04	1.34	1.49	1.66	1.93
Average	0.95	0.83	0.84	0.87	0.89	0.99	0.92	1.15	1.49	1.38
<i>Fructosan</i>										
6/6/35	2.34	2.73	2.11	1.42	0.93	2.51	1.99	2.34	1.64	1.25
25/6/35	0.69	0.93	0.81	0.31	0.28	2.36	1.95	2.67	1.38	1.95
9/7/35	0.12	0.00	0.11	0.00	0.12	1.16	2.28	2.45	1.66	1.22
Average	1.05	1.22	1.05	0.58	0.44	2.01	2.07	2.49	1.56	1.47
<i>Sucrose</i>										
6/6/35	0.96	0.92	0.73	0.76	0.74	0.67	0.91	1.35	1.01	0.99
25/6/35	0.66	0.94	0.80	0.40	0.77	0.93	1.00	0.91	0.79	0.63
9/7/35	0.73	1.43	1.12	1.45	1.62	1.65	1.80	1.74	1.71	1.62
Average	0.78	0.76	0.88	0.87	1.04	1.08	1.23	1.33	1.17	1.08

TABLE V

Reducing Sugar, Fructosan, and Sucrose Contents of Barley Leaves, Stems, and Ears from Plants grown in Sand and receiving Nitrogen-deficient Dressings (see Part I, p. 185).

Results are means of duplicate analyses and expressed as percentage of the fresh weight.

Date of collection.	Leaves.		Stems.		Ears.	
	control.	—N.	control.	—N.	control.	—N.
<i>Reducing sugars</i>						
8/7/37	0.53	0.35	0.94	0.47	0.56	0.78
15/7/37	0.94	0.41	1.04	0.61	2.40	2.04
26/7/37	0.41	0.39	0.91	0.32	1.65	1.23
Average	0.63	0.38	0.96	0.47	1.54	1.35
<i>Fructosan</i>						
8/7/37	0.16	1.78	0.30	4.90	2.11	4.39
15/7/37	0.09	1.03	0.51	3.20	2.98	1.39
26/7/37	0.14	0.66	0.50	4.04	1.56	1.64
Average	0.13	1.15	0.43	4.03	2.22	2.47
<i>Sucrose</i>						
8/7/37	1.10	1.93	0.57	0.72	0.88	0.74
15/7/37	0.33	1.51	0.34	1.33	1.03	1.15
26/7/37	0.77	1.10	1.01	1.73	1.20	1.09
Average	0.73	1.51	0.64	1.26	1.03	0.99

(loc. cit.) for barley leaves that a low level of nitrogen manuring not only raises the level of total sugar but also raises the ratio of sucrose to reducing sugar. Further, it is now shown that this same effect extends to the stems, and there is an additional increase of fructosan. Lowering the nitrogen level thus appears to exert a dual effect: first, it produces an increase in the level of soluble sugars, presumably owing to the shortage of nitrogen for protein synthesis, and secondly, an alteration of the relative amounts of the different sugars present in the direction of less reducing sugar and more sucrose and fructosan. The effect on the fructosan content is apparent in both the plants grown in soil and those grown in sand, but the alteration of the ratio of sucrose to reducing sugar was not apparent in the soil cultures grown at Slough (Table IV). In the plants grown at Rothamsted in soil, however, the level of reducing sugar in both leaves and stems was very high in 1935, when the nitrate dressing was given, while in 1936, in the absence of added nitrate, the reducing sugar level was low throughout, although the total sugar stored was greater than in 1935 (see Table II).

Analysis of the ears from the sand-culture experiment brought out the interesting point that the composition of the ears with respect to sugar content was little affected by the treatment.

Seasonal drift of dry weight, and water-insoluble material of leaves, stems, and ears.

The fresh weights, dry weights, and material insoluble in water and 95 per cent. alcohol are shown in Table VI in terms of the weight of each fraction in twenty shoots. In the leaves, dry weight and insoluble material both increase during leaf development, and the proportion of insoluble material rises a little from 55 to 65 per cent. of the dry weight. Following full expansion of the last leaf, the amounts remain constant for a short time and then fall slowly, and the proportion of insoluble material tends again to fall. The general drift is thus similar to that described for total sugar. In terms of concentration there is a rather marked rise after stem elongation is complete, but the figures of Table VI, calculated in terms of weight per shoot, show that this rise is due to water loss and not to increasing dry weight.

In the stems dry weight and insoluble material rise continuously until stem elongation is complete, and again there is little variation in the ratio of the two quantities. The value in the stems increases from 60 to 70 per cent., and is thus somewhat higher than in the leaves. It seems likely that the maximum value for the insoluble fraction was reached about July 10, in spite of the rather high value recorded for the final collection on July 27. In the ears dry weight and insoluble material also rose continuously, the proportion of insoluble material being about 70 per cent.; in this case starch present will be included in the insoluble fraction.

The effect of defoliation and ear removal on the dry weights and insoluble material is shown in Table VII. In the defoliated samples dry weight of the

TABLE VI

The Distribution of 'Carbohydrates' other than Soluble Sugars in Leaves, Stems, and Ears of Barley during Growth. Fresh Weight (F.W.), Dry Weight (D.W.), Material insoluble in Cold Water and 95 per cent. Alcohol (R), Soluble Material other than Sugars (s), Estimate of Insoluble Polysaccharides (X), and of Soluble Material of Unknown Composition (Y). (See text for method of obtaining columns X and Y.)

Date.	Whole shoots.				Leaves				Stems.				Ears.								
	F.W.	D.W.	F.W.	D.W.	R.	s.	X.	Y.	F.W.	D.W.	R.	s.	X.	Y.	F.W.	D.W.	R.	s.	X.	Y.	
1936.																					
12/5	7.5	0.91	4.48	0.65	0.35	0.26	0.13	0.09	2.37	0.26	0.15	0.10	0.09	0.03	—	—	—	—	—	—	—
19/5	18.3	2.51	12.02	1.81	1.02	0.60	0.35	0.16	6.35	0.70	0.46	0.20	0.30	0.01	—	—	—	—	—	—	—
26/5	34.5	4.76	21.60	3.35	1.89	1.20	0.85	0.32	12.92	1.41	0.88	0.46	0.63	0.08	—	—	—	—	—	—	—
3/6	51.0	6.79	27.0	4.10	2.35	1.39	1.24	0.50	24.0	2.69	1.54	1.01	1.11	0.00	—	—	—	—	—	—	—
9/6	71.0	9.95	35.5	5.96	3.18	2.12	1.85	0.87	35.5	3.99	2.40	1.24	1.98	0.31	—	—	—	—	—	—	—
16/6	100.0	13.62	45.0	7.65	4.53	2.21	3.16	0.73	54.0	5.97	3.89	1.34	3.29	0.00	—	—	—	—	—	—	—
19/6	115.0	15.96	50.0	8.75	5.30	2.45	3.85	0.81	63.0	7.21	4.97	0.74	4.33	0.00	0.6	—	—	—	—	—	—
22/6	130.0	19.25	54.0	9.42	5.84	2.83	4.30	1.05	72.0	9.83	6.88	1.74	5.84	0.00	1.6	—	—	—	—	—	—
24/6	135.4	21.68	58.0	9.85	6.48	2.36	5.44	0.59	75.0	11.83	8.21	2.09	7.62	0.20	3.4	—	—	—	—	—	—
26/6	156.0	27.25	56.2	10.73	8.02	1.74	7.26	—	92.5	15.29	—	—	—	—	7.3	1.23	0.62	0.17	0.50	0.08	
29/6	182.5	31.74	76.8	13.36	8.29	4.17	7.41	1.84	95.1	16.28	11.10	2.35	10.29	0.40	10.6	2.10	0.99	0.53	0.78	0.37	
1/7	167.3	30.01	59.5	9.63	5.97	3.09	5.21	1.46	99.6	16.91	12.46	2.42	11.70	0.44	17.2	3.47	2.36	0.44	2.08	0.13	
3/7	197.9	38.38	55.4	11.78	7.45	3.57	6.63	1.60	113.1	20.36	15.19	2.84	14.38	0.50	29.5	6.24	4.51	0.67	3.96	0.05	
6/7	187.3	44.94	50.4	13.77	7.31	—	6.35	—	111.0	24.05	16.45	3.69	15.49	1.05	25.9	7.12	5.18	1.03	4.55	0.33	
10/7	236.6	53.58	59.5	12.67	7.80	3.94	6.63	1.79	133.3	29.34	20.19	4.61	19.02	1.54	43.9	11.57	8.35	2.01	7.33	0.80	
14/7	188.3	60.60	47.6	13.05	7.70	—	6.74	—	115.7	32.30	21.27	5.03	20.30	2.81	45.9	15.25	10.67	2.74	9.33	1.14	
17/7	231.5	63.62	57.5	13.50	8.17	4.11	7.15	1.95	118.1	34.34	20.22	6.05	19.00	3.69	55.9	15.78	12.81	—	11.42	—	
21/7	199.0	68.15	33.7	10.61	6.44	2.94	5.41	1.40	103.9	34.61	21.79	4.75	20.75	2.37	60.7	22.93	16.25	3.37	14.23	0.97	
27/7	233.0	86.95	33.5	11.38	6.59	3.38	5.27	1.74	117.7	44.21	27.44	4.58	26.11	1.55	81.8	31.36	21.48	5.26	18.87	1.97	

stems continued to increase, but very slowly. There must thus have been either translocation of dry matter from the roots or some photosynthetic activity in the stem itself. In the last two samples (14/7 and 17/7) the values of both dry weight and insoluble material were particularly high, and there was a sudden increase in the dry weight of the ear at the same time. This increase was associated with a rapid emergence of the ears after an apparently static period following the defoliation. On 17/7 the dry weight of the ears was about half that of the control plants.

TABLE VII

Dry Weight (D.W.) and Material Insoluble in 95 per cent. Alcohol and Water (R) in Barley Plants. F, Plants defoliated 24/6/36; E, Ears removed 29/6/36. (Control Samples in Table VI.)

All results stated as gm. per 20 shoots.

Date 1936	Leaves.		Stems.				Ears.	
	E.		F.		E.		F.	
	D.W.	R.	D.W.	R.	D.W.	R.	D.W.	R.
26/6	.	—	12·78	9·38	—	—	1·12	0·60
29/6	.	—	12·26	9·88	—	—	1·12	0·71
1/7	.	11·91	13·43	10·78	21·43	16·09	2·06	1·37
3/7	.	13·34	12·85	9·67	24·72	17·26	2·00	1·90
6/7	.	12·63	13·77	12·35	26·63	16·79	2·75	1·60
10/7	.	13·88	15·45	10·65	28·05	16·13	3·74	2·77
14/7	.	12·80	18·80	13·56	32·00	18·67	7·09	5·19
17/7	.	13·31	17·81	13·43	26·52	15·95	7·55	5·59
21/7	.	13·25	—	—	36·37	22·14	—	—
27/7	.	12·69	—	—	35·47	21·24	—	—

Ear removal resulted at first in little change in the dry weight of the leaves, but appeared to check the fall noted later in the control plants. In the stems there was a relatively large increase in dry weight, all in soluble material. The insoluble fraction in fact tended to be lower than in the controls, and it is therefore concluded that the restriction of growth inhibits the formation of polysaccharides and that soluble sugars delivered from the leaves accumulate instead.

Relationship of sugar content to total carbohydrate.

The insoluble fraction discussed above will consist in the main of structural carbohydrates and proteins. It has been possible to make an approximate estimate of the protein present from records (unpublished) of the nitrogen and ash contents of barley leaves, stems, and ears collected by Gregory during four seasons from plants grown in soil. By subtracting these estimates from the figures of column *R*, a measure of polysaccharide has been obtained and is entered in columns *X* in Table VI. Variations in this fraction are discussed below, together with those of the soluble carbohydrates.

Little attention has hitherto been paid to quantitative exchanges in soluble material other than sugar, and in a few instances organic acids, during the growth of plants. Barnell (1936) determined the seasonal drift of alcohol-soluble material in winter wheat. He found that sugar formed less than half of the soluble fraction and that the general drift of the undefined portion was similar to that of sugar. From the data presented here the amount of water- and alcohol-insoluble material, other than sugar, has been found as the difference between the total dry weight values and the sum of the insoluble material and the total sugar (Table II) for the 1936 series, and is entered in columns *s* in Table VI. These observations, which are of a preliminary nature, have been made so that changes in this fraction possibly associated with sugar changes should not be overlooked. Since this fraction will include fats, mineral constituents, and soluble nitrogenous compounds, an attempt has been made to allow for these substances. Fats have been determined in some of the samples as the ether-soluble fraction. The average values found were for the leaves 7 per cent., for stems 6 per cent., falling to 2 per cent. with increasing age, and for ears 4 per cent. of the dry weight. Approximate values for soluble nitrogen (assuming 20 per cent. of the total is soluble) and for ash were calculated from Gregory's data. The residual soluble material remaining after subtracting these three fractions is entered in columns *Y* in Table VI.

In the leaves the *Y* fraction is throughout of the same order as the total sugar content, and in old leaves may even be greater. The maximum amount was about 15 per cent. of the dry weight. In the stems there is no evidence of a *Y* fraction until rapid stem elongation begins, when it appears and reaches a final value equal to about one-third of the sugar content, or 10 per cent. of the dry weight. In the ears there is also an accumulation as growth proceeds.

The presence in the leaves of an undefined soluble fraction equal in magnitude to that of sugar, and occurring to a lesser extent in the stems, may clearly repay further study, especially as accumulation of this fraction runs parallel to that of sugar in the leaves (cf. Barnell, loc. cit.). Hydrolysis of leaf extracts with N acid (see Part I, p. 194) suggests that in the leaves further carbohydrate material may be present which is not included in the present estimates of soluble sugars, based on hydrolysis by N/5 acid. This possibility is now being examined. Similar hydrolysis of stem extracts has so far failed to give any indication of the presence of additional carbohydrate here. It is obvious that the inclusion of estimates of organic acids is urgently called for.

The very approximate nature of these estimates of undefined soluble material is fully realized, particularly the fact that no part of the mineral content has been assigned to the insoluble fraction. This may be a serious omission in view of the high percentage of silica in the ash of cereals. Such an allowance would, of course, merely increase the estimate of the undefined fraction.

The carbohydrates accumulating during growth may thus be divided into

an insoluble fraction (X) (cellulose, hemicellulose, and starch if present), soluble sugars (S_T), and part of the soluble fraction (Y). In the following paragraphs the term 'carbohydrate' is used to denote the sum of these three fractions. The course of change of X , Y , and S_T is shown in Fig. 9, and it is abundantly evident that throughout the growth cycle the principal feature of carbohydrate metabolism is the synthesis of polysaccharides either in leaves, stems, or ears. The soluble sugars represent only a balance remaining after synthetic and translocatory activities are fulfilled and do not appear to exert a determining influence on these processes; the amount of sugar is rather determined by the rates of such processes relative to photosynthesis. During the period of maximum leaf development sugar only accumulates in the leaves in sufficient quantities to maintain a concentration of 1 to 3 per cent. of the fresh weight, and in the stems the concentration is still lower at this stage; rate of synthesis of polysaccharide in the leaves and stems therefore keeps pace with the sugar formation by photosynthesis. When polysaccharide synthesis ceases in the leaves, at the time of full expansion of the last leaf, relatively more sugar is translocated and accumulation begins in the stems. Finally, when stem growth is complete the stored sugar may increase to a considerable extent. As the leaves die off it is probable that the removal of sugar to the ear will exceed the increase in the stems and stem sugar will fall, but at first the demands made by the growing ear are less than the supply from leaves to stems.

In the absence of analyses of the roots it is not yet possible to present a complete picture of the carbohydrate distribution in the barley plant. From the present data the distribution in leaves, stems, and ears is summarized in Table VIII for four stages of the growth cycle, development of leaves 1 to 5, development of leaves 5 to 10, stem elongation, and ear growth when stem growth is complete. The percentage of the total increase in carbon compounds (i.e. dry weight less ash) appearing as 'carbohydrate' is shown in the second column. In the first stage only 54 per cent. appears in this form, an expression also of the well-known fact that young plants utilize large amounts of carbon in protein synthesis. The proportion appearing as 'carbohydrate' increases at the later stages to 91 per cent.

Of 2.13 gm. of 'carbohydrate' accumulated during development of leaves 1 to 5, 38 per cent. appears as insoluble fraction in the leaves and the remainder is presumably primarily stabilized in a soluble form, largely as sugar. 15 per cent. remains in the leaves as the Y fraction and 11 per cent. as sugar, while the remaining 36 per cent. is translocated and appears almost entirely as polysaccharide in the stems (leaf sheaths), there being only 4 per cent. of sugar. At this stage sugar only represents 15 per cent. of the total material involved in carbohydrate changes. 11.5 gm. of 'carbohydrate' appeared during the development of leaves 5 to 10, and again a very high proportion was polysaccharide. The proportion translocated rose to 58 per cent., of which 46 per cent. was synthesized to polysaccharide. During stem elongation

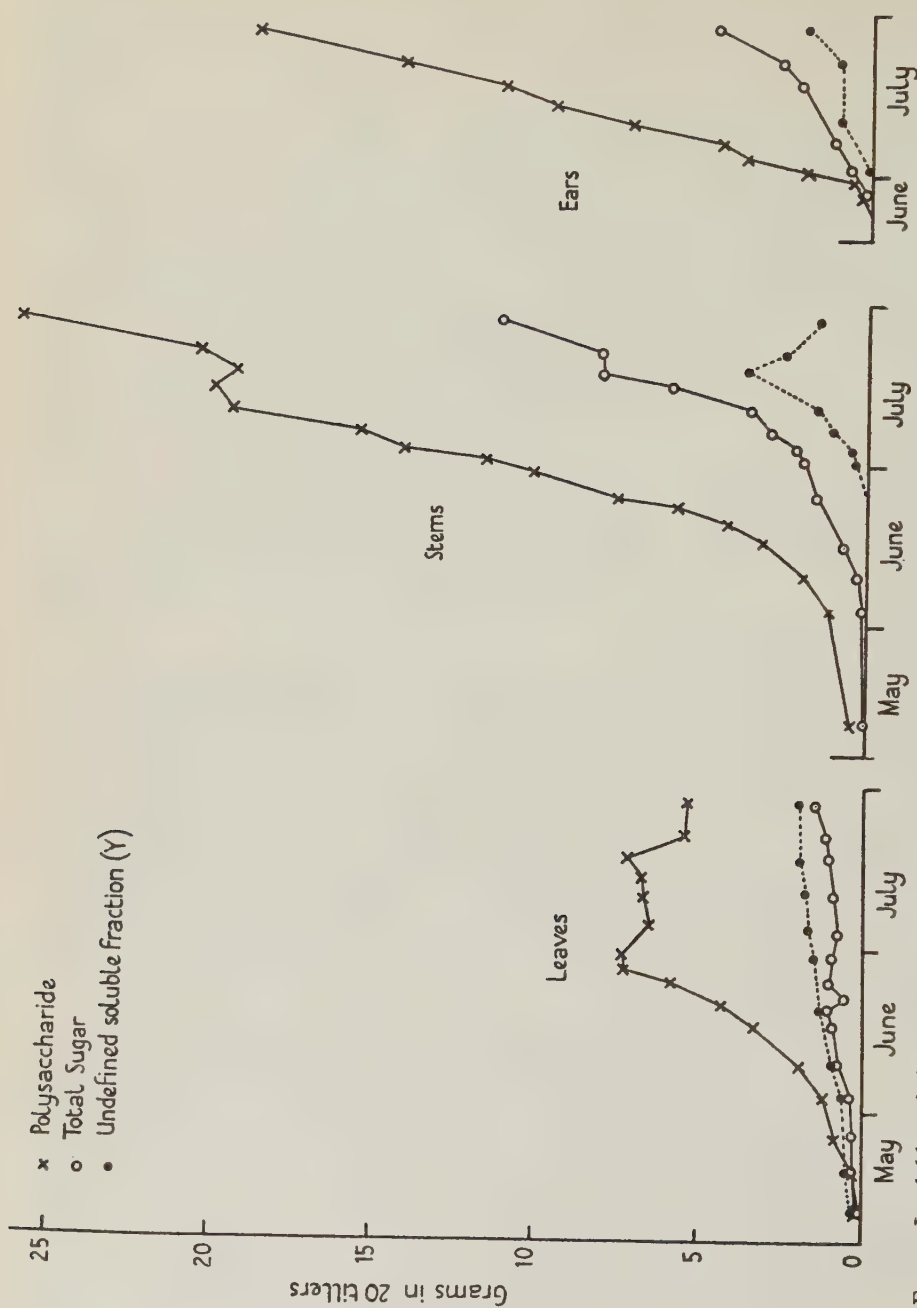


FIG. 9. Insoluble carbohydrate, total sugar, and undefined soluble material (grammes in 20 shoots) in leaves, stems, and ears of barley during growth, 1936. (The calculation of estimates of insoluble carbohydrate and undefined soluble material is described in the text.)

TABLE VIII

Distribution of Assimilated Carbon as 'Carbohydrate' in Leaves, Stems, and Ears of Barley during Growth. (The Undefined Water-soluble Fraction is included in the 'Carbohydrate'.)

During growth of:	Date 1936.	Increase in carbon compounds (D.W. — Ash), gm. in 20 shoots.	Increase in 'carbohydrate' as per cent. of carbon compounds.	Increase in 'carbohydrate'.			Translocated to stems and ears.	Increase in 'carbohydrate' in:						
				Leaves				Stems		Ears		Insol. carb.	Sol. sugs.	Undefined (Y).
				Insol. carbohydrate.	Sol. sugs.	Undefined (Y).		Insol. carb.	Sol. sugs.	Undefined (Y).				
percentage of the 'carbohydrate' increase														
Leaves 1-5 Leaves 5-10 Stems Ears	germination to 26/5	4.10	54	38	11	15	36	29	4	—	—	—		
	26/5-22/6	12.99	89	29	7	6	58	46	12	0	—	—		
	22/6-10/7	33.21	88	8	0	3	89	45	10	4	24	3		
	10/7-21/7	14.49	97	-9	2	-2	109	12	25	13	48	11		

89 per cent. was translocated, growth of the leaves being nearly complete, and this material was divided between the stems and ears, 69 per cent. as polysaccharide and the rest as sugar, and the Y fraction. Finally when stem growth is complete soluble sugars become an important fraction of the 'carbohydrate' increase, namely, 38 per cent. At this stage leaf dry weight falls so that the increase in stems and ears is 109 per cent. of the total, of which 50 per cent. is in the stems and 59 per cent. in the ears.

DISCUSSION

The position of fructosan in relation to other sugars, as shown by the results presented here, entirely supports the view that this sugar is of secondary origin and arises from reducing sugars rather than directly from sucrose. Thus in leaves, under normal conditions of growth, fructosans are present in small amounts only and show no important fluctuations. When, however, conditions are operative which limit the demand for soluble sugars in other parts of the plant some accumulation of fructosan in the leaf results. Such conditions may be either nutritional, for example, lack of nitrogen, or may be produced by removal of tillers or ears from the plant. Alternatively, when sugar accumulation is reduced by a high level of nitrogen manuring fructosan is absent or only present in traces. Whether there is a diurnal fluctuation in fructosan content is not known, and it does not at present seem profitable to draw any analogy with starch in other plants. In the barley leaf, in common with many others, sucrose is always present in excess of all other sugars, and in the very early leaves it is doubtful if any other sugar is present at all. Sucrose changes in the leaf are thus always dominant, and changes in fructosan and reducing sugar relatively small.

Since the principal sugar stabilized in the leaves is sucrose, it seems reasonable to accept the view of Mason and Maskell (1928) that whatever the first sugar of photosynthesis may be, sucrose is initially translocated from the leaf parenchyma. It has been found that the barley leaf contains a highly active water-soluble invertase, and therefore inversion of sucrose may well begin in the leaf itself and a mixture of sucrose and reducing sugar be delivered to the stems. If this were the only change in sugar not utilized at once for polysaccharide synthesis, an increasing concentration of reducing sugar from apex to base would result, while actually the reverse is found. There is, however, an increasing concentration of both sucrose and fructosan from apex to base, suggesting that fructosan is produced from inverted sucrose. During the growth of any internode sugar is supplied by the leaf attached to the node above and by parts of the stem above, and fructosan, sucrose, and reducing sugars all increase. When growth ceases and the attendant leaf begins to die the sugar now supplied only by the parts of the stem above is stored solely as fructosan. During this process of translocation from the stem above, therefore, sugar is all converted to fructosan. If the upper leaves remain green for some time after stem elongation is complete (as in the 1936 experiment) there

may be considerable storage of sugar in the stems, translocation to the ear being less rapid than that from the leaves. In this case the uppermost internodes, receiving sugar direct from the remaining leaves, have the highest proportion of sucrose, but throughout there is a continuous increase of fructosan. If the leaves die off more quickly (as in 1935) a fall in sugar content of the stem occurs as soon as growth is complete, and the additional storage of fructosan is much reduced. Fructosan formation thus appears to be a relatively slow process undergone by temporarily immobilized sugar.

When there is a demand for sugar at new growing-points fructosan is readily re-utilized as is shown by its disappearance along with other sugars in the defoliation experiment. The high concentration of this sugar in the peduncles of normal plants and its appearance in the early stages of development in the ear suggest that fructosan itself is translocated, and conversion to other substances can only take place at growing-points. The source of fructosan in the stems may be both the inversion products of sucrose, involving a conversion of glucose to fructose, or perhaps fructose remaining after utilization of glucose in polysaccharide synthesis, by preferential transference to ears or roots, or in respiration alone, acts as the precursor of fructosan. From considerations put forward below the utilization of fructose rather than of glucose in respiration appears at present the simplest hypothesis. There is no evidence of preferential transference of glucose to the ears, since large amounts of fructosans are found in both peduncles and ears; moreover, the greatest accumulation of fructosan in any internode occurs when its growth (i.e. polysaccharide synthesis) is complete. There remains the possibility of preferential transference of glucose to the roots. Russell (1937) has found that with some manurial treatments fructosan may be absent from barley roots, so that the possibility that sugar translocated to the roots is largely glucose cannot be excluded. On the whole, however, it would seem probable that all the sugar entering a fully grown internode may be converted to fructosan and that conversion of glucose to fructose does occur.

Further consideration of the origin of fructosans and the possible inter-conversion of glucose and fructose must depend on a knowledge of the relationships of the soluble sugars to the general carbohydrate metabolism. Since a very high proportion of the carbohydrate is present as polysaccharide (cellulose, &c.), the principal feature of that aspect of metabolism involving the conversion of photosynthesized sugar is obviously polysaccharide formation. Moreover, it is well known that in the vegetative stage of growth a large proportion of assimilated carbon is used in protein synthesis, and in formulating any satisfactory picture of carbohydrate changes this synthesis must be taken into account. In the early stages of growth the sugar concentration is low, and at first as much as 70 per cent. of the assimilated carbon may take part in protein formation and most of the remaining 30 per cent. in polysaccharide formation. As the vegetative stage nears completion the proportion of polysaccharide in relation to protein formed increases, and there is a small

increase in the soluble sugars. When organization of the stem internodes occurs the renewed protein metabolism is masked by the large production of mechanical tissue, and finally protein synthesis in the ear after fertilization is again overshadowed by the polysaccharide synthesis leading to the development of the endosperm and storage of starch. Only during the stage of ear development following stem elongation is there any important accumulation of sugar when considered in relation to the amount of polysaccharide and protein synthesis. It seems clear that at all stages of growth available sugar is used first for protein synthesis and secondly for polysaccharide synthesis, and that the free-sugar level at any time represents only the balance of the supply over the local requirements for growth purposes. Accumulation in the stems therefore depends on the relative rates of sugar translocation from the leaves and to the ears, and may be much reduced or quite suppressed by curtailment of the sugar supply without preventing ear development. This is shown in the 1935 experiment, where the leaves died rather soon, and in the defoliation experiment. In the former case ear development proceeded normally, with rapid depletion of the stem sugar, and in the latter both stems and ears maintained a restricted growth in the virtual absence of free sugar. The synthesis of polysaccharide in the stems and ears was thus in no sense dependent on accumulation of any critical concentration of sugar in the stem. If, on the other hand, the demand for sugar is cut off by removal of the ears, since photosynthesis is not prevented there is an immediate increase of sugar in the leaves themselves, and more particularly in the stems, and a large part of this immobilized sugar is fructosan.

In addition to producing a large increase in soluble sugar, ear removal led to reduced stem growth (i.e. polysaccharide synthesis), while vegetative growth was renewed by a fresh production of tillers. One factor controlling the polysaccharide formation associated with stem elongation may thus be the presence of a regulating substance produced in the ears, and in its absence there is a reversion to vegetative growth. Stem growth may therefore occur in the absence of stored sugar, as in the defoliated plants, and may be inhibited in the presence of large amounts of sugar as after ear removal. It is once more evident that different sugar-levels are only the result of variation in the relation of sugar supply to protein and polysaccharide synthesis. High sugar-levels produced by restriction of these activities favour the formation of fructosans.

From the chemical point of view it is not yet possible to form a clear-cut picture of the interrelationships of glucose and fructose, but such conclusions as can be drawn from the present data on the whole support the view that if sucrose is the starting-point for synthesis, some conversion of fructose to glucose must occur at least in the stems. If, over any given period, increase of polysaccharide, free glucose, and glucose combined as sucrose be taken as a measure of the amount of glucose involved in metabolism, it follows that an equal amount of fructose must have been used either in respiration or

in synthesis, if no conversion to glucose occurs. Assuming that carbon compounds not specified as 'glucose' are derived from fructose, a minimum value for the amount of fructose which would have to be respired can be found as the difference between twice the 'glucose' and the total increase in dry matter (less ash), and a maximum estimate by assuming that fructose does not take part in synthesis and only appears as fructosan, free fructose, or combined in sucrose. From the data of Table II such calculations have been made for the leaves and stems for the four intervals given in Table VIII, using the appropriate hours of darkness for each period. In the leaves the limits calculated as CO_2 were 0.30 and 0.76 mg. per gm. fresh weight per hour. Gregory and Baptiste (1936) quote an average rate of respiration of 0.50 mg. per gm. fresh weight per hour. In leaves therefore no conversion of fructose to glucose is necessary to account for the production of polysaccharide, provided fructose is the sole source of carbon for respiration and contributes largely, if not entirely, to the synthesis of proteins, fats, &c. In the stems the limits were 0.54 and 2.00 mg. per gm. fresh weight per hour. These values are too high to be acceptable and in addition would require a loss in respiration of nearly half the dry-weight increase. In the stems, therefore, conversion of fructose to glucose seems essential, unless at the stage of active stem growth fructose and not glucose is preferentially translocated to the roots, a possibility which, it has already been pointed out, is unlikely.

The general conclusion to be drawn seems to be that conversion of fructose (produced from inverted sucrose) into glucose may occur at the seat of rapid polysaccharide synthesis, that is, at points of active growth, whereas, when sugar is stored in tissues which are not growing there is a tendency for the process to be reversed and glucose to be converted to fructose (or fructosan). Without definite evidence that fructose is actually used in protein synthesis and as the chief respiratory substrate such a conclusion must be regarded as very tentative, since many cases are known of the ready utilization of fructose in starch formation, and it may also be the precursor of cellulose complexes (Butlin 1936). It cannot therefore be assumed that fructose is not used in polysaccharide synthesis while glucose is respired.

SUMMARY

1. The seasonal drift of fructosan, sucrose, and reducing sugars in the leaves, stems, and ears of barley plants grown in soil were studied during two seasons, together with the effect of defoliation, ear removal, and of nitrogen deficiency on the sugar content.

2. In the leaves sucrose was throughout the predominant sugar, and reducing sugars and fructosan showed no important changes. Sugar content of the leaves rose until the last leaf expanded, and fell slowly during stem elongation and ear development.

3. Fructosans were always present, though in small amounts, in leaves of

plants grown under normal conditions. Removal of some tillers or of the ears, resulted in an increase in fructosan, as also did nitrogen deficiency, while a high level of nitrogen reduced fructosan formation. It is concluded that fructosans are of secondary origin in the leaves and arise when the demand for soluble sugars in other parts of the plant is reduced.

4. In the stems the proportion of sucrose was lower and of fructosan and reducing sugar higher than in the leaves. There was a fall in reducing-sugar concentration and a rise in sucrose and fructosan concentration from apex to base; fructosan therefore appears to be formed from reducing sugar produced by inverting sucrose and accumulates in the stems as growth is completed.

5. The greatest accumulation of fructosan in any internode of the stem occurs after its growth has ceased, and it therefore seems improbable that fructosans arise as a result of fructose condensation alone, the glucose being simultaneously used for polysaccharide synthesis. The possible accumulation of fructosans as a result of preferential translocation of glucose away or loss of glucose in respiration is discussed. It appears most likely that fructosans are formed from invert sugar and require a conversion of glucose to fructose.

6. Defoliation resulted in an immediate loss of all the sugar in the stem, but restricted growth of both stem and ear continued. Ear removal also resulted in restricted growth of the stem and a considerable accumulation of soluble sugars, particularly of fructosan. Sugar-level thus exerts no simple determining influence on polysaccharide formation in the stem, but is rather the result of restriction of growth by other causes.

7. High concentration of fructosans occur in the peduncles and in the young ears; these sugars therefore are translocated to the ear and only re-utilized at active seats of polysaccharide synthesis.

8. The relation of soluble sugars to total carbohydrate metabolism is discussed, and it is suggested that if the starting-point of polysaccharide synthesis be sucrose, in the leaves no conversion of fructose to glucose need be postulated to account for the cellulose, &c., formed, provided fructose is the source of carbon for respiration and the synthesis of proteins, &c. In the stems, however, such a conversion is necessary to account for the formation of the large amount of mechanical tissue, unless there is translocation of excess fructose to the roots. Since fructosans are not always found in roots this hypothesis is considered as unlikely.

9. It is concluded that fructosan formation is a relatively slow process undergone by temporarily immobilized sugar and that its formation probably necessitates a conversion of glucose to fructose. On the other hand, when the demand for sugar is renewed at active growing-points the reverse change of fructose to glucose occurs, after translocation of the fructosan to the growing-point.

10. It is shown that in the leaves the amount of undefined water- and alcohol-soluble material equals that of total sugar, and in general fluctuates in the same way. Part at least of this material yields reducing sugar on

hydrolysis with strong (N) acid. In the stems the amount undefined is much less, and no evidence of a further source of sugar was found.

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A Comparative Physiological Study of Sugar-beet and Mangold with respect to Growth and Sugar Accumulation

I. Growth Analysis of the Crop in the Field

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With fourteen Figures in the Text

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INTRODUCTION

A SERIOUS difficulty encountered in any investigation of the changes during growth of a field crop is the high variability between plants of the crop. To measure and control this variability, all observations must be made on a number of samples drawn at random from the whole population, so that the labour involved in making even a simple measurement is considerable. Less variable material can be obtained by using pot-cultures, but the conditions of pot-culture differ so widely from field conditions that the results must be used with caution in interpreting the behaviour of plants growing in the field.

Simple measures of growth such as counts of plants and shoots and measurements of shoot length have been much employed in work on cereals. For some purposes, such as the establishment of empirical relations between final yield and growth at stages previous to harvest from which forecasts of yield may be made, these measurements may be useful, but they are of little fundamental physiological significance, for the changes which they measure are morphological functions of the whole complex of physiological processes. Nor are they easily adaptable to crops such as sugar-beet in which the form of the plant changes little during growth.

For a physiological approach to the study of growth it is essential to use the more fundamental measures of change in dry weight, in leaf area, and in composition. Gregory (1917) and West, Briggs, and Kidd (1920) have developed methods of growth analysis which make use of growth functions of definite physiological significance, in particular, the leaf area of the plant and the rate of increase of dry weight per unit leaf area, which measure the size of the assimilatory system and the intensity of assimilation respectively. Notwithstanding the fact that these methods were developed long ago, not many investigators have attempted growth analysis of field crops. The following instances may be cited, Tincker and Jones (1931), Crowther (1934, 1937), Heath (1937).

The sugar-beet plant presents many problems of great physiological interest. The development and maintenance of a high sucrose content in the root is the most important feature of the plant agriculturally, but little is known of the physiological factors involved. Field experiments carried out over a period of years have shown that the effect of fertilizers on the crop is usually small but varies widely with soil and season. The yields obtained in Great Britain are generally lower than on the Continent, and this together with the small effect of added mineral nutrients suggests that the growth of the crop in this country is limited by some suboptimal climatic factor.

The present work was begun as a preliminary to the investigation of these problems. Its object was to measure in as great detail as possible the changes taking place during the growth of a normal crop of sugar-beet, in particular the variation with time of dry weight, water content, and sugar content of all

parts of the plant, and also of leaf number and leaf area, and to correlate these changes as far as possible with meteorological factors.

In order to discover whether the characteristic high sugar content of the sugar-beet is related to some special feature of growth, the experiment was arranged so that a comparison could be made with mangold grown in comparable conditions. Sugar-beet and mangold are usually regarded as varieties of the same species and the mangold was used as a control plant, being closely related to the sugar-beet but more physiologically normal. The comparison of the two crops is of some practical interest, for it has been suggested (Rothamsted Annual Report, 1932) that a greater yield of sugar per acre can be obtained from mangold than from sugar-beet. This is based on a comparison of average yields and sugar contents, and the direct comparison in an experiment has not previously been made.

Sugar-beet, as normally grown, is a biennial plant harvested in the first year of its growth while still in a vegetative state and not at a clearly defined stage of maturity. Consequently the length of the growth period, which may be varied by varying the date of sowing, is likely to be of even greater importance in determining the final yield than in crops such as cereals where the ripened fruit is harvested. Plants sown on different dates will reach the same stage of growth at different times in the growing season. The same seasonal changes in climatic factors operating at different stages in the growth cycle may produce different quantitative and qualitative effects on growth. It was possible to investigate such effects without increasing the size of the experiment or affecting the accuracy of the sugar-beet and mangold comparison by sowing the replicates on successive dates. In addition to providing a measure of possible sowing-date effects, this arrangement was advantageous in that the comparison of sugar-beet and mangold was made on a wider basis, and was not specific to one sowing date.

ARRANGEMENT OF EXPERIMENTAL PLOTS

The experimental area was divided into six blocks each consisting of two plots, one of which, selected at random, was sown with sugar-beet, variety Kleinwanzleben E, and the other with mangold, variety Garton's Yellow Globe. The blocks were sown at intervals of a fortnight, one at each sowing date, beginning on April 9, 1934. The sowing date treatments were applied to blocks at random.

The dates of sowing were:

	Weeks after sowing block I.		Weeks after sowing block I.
I. April 9	0	IV. May 21	6
II. April 23	2	V. June 4	8
III. May 7	4	VI. June 18	10

The experimental area had been cropped in 1933 with spring oats, and had received a dressing of $1\frac{1}{2}$ cwt. of sulphate of ammonia per acre.

The following quantities of fertilizers were applied to every plot: 0.6 cwt. N per acre as sulphate of ammonia, applied at sowing, 0.5 cwt. P_2O_5 per acre as superphosphate and 1.0 cwt. K_2O per acre as 30 per cent. potash salts applied on April 23. The area of each plot was 0.35 acre. The rows were spaced 22 in. apart, and the plants were thinned to approximately 9 in. apart in the rows. There were nine rows of plants per plot, of which the two outside rows were not sampled; the end plants of each row were also rejected.

Table I shows the dates on which the plots were thinned, and the mean number of leaves per plant greater than 1 in. in length, including the cotyledons, at the time of thinning.

TABLE I

Date of sowing.	Date of thinning.	Days after sowing.	Mean number of leaves per plant.	
			Sugar-beet.	Mangold.
I	May 30	51	6.8	7.5
II	June 12	50	7.5	7.7
III	June 15	39	7.7	7.7
IV	July 11	51	6.7	6.5
V	July 16	42	6.8	6.7
VI	July 18	31	5.9	6.1

METHODS

1. *Sampling procedure.*

Sampling began when the plants were thinned, and continued at fortnightly intervals. On the day before thinning, the plants had been 'bunched', leaving groups of plants which were thinned to single plants in the final operation. The samples at thinning consisted of one plant taken at random from every fifth bunch of the plot.

All the later samples consisted of twenty plants taken from each plot. Three plants were taken at random from each of the seven centre rows of the plot, with the exception of one row, selected at random from the seven, from which only two plants were taken. Only normally spaced plants were included in the samples. Plants adjacent to a gap in the row, where a plant was missing either through thinning irregularities or because it was taken in a previous sample, were rejected. It was found impossible to deal with the samples from all twelve plots in one day, so that on each sampling occasion the plots of the first three sowing dates were sampled on the first day, and the remaining three on the second day. The sugar-beet and mangold plots of the same sowing date were sampled together, but the order of sampling of the three sowing dates taken on any one day was determined at random afresh at each sampling time. The plants were dug with a fork, and in the sugar-beet care was taken to remove the whole of the storage region of the root.

The procedure of sampling was as follows: at about 10.0 a.m. the first two samples from a sugar-beet and a mangold plot of the same sowing date were dug up and removed to a field laboratory, where the various weighings and

measurements were carried out. When these were completed at about 12.30 p.m., the next two plots were sampled, weighed, and measured, and the remaining two samples were taken at about 4.0 p.m. This method ensured that comparisons between sugar-beet and mangold were independent of time of day of sampling, but comparisons between sowing dates involved differences between times of day and also between the first and second day of sampling. The only results likely to have been affected seriously by this unavoidable spread of the observations over two days are the carbohydrate estimations on leaf lamina and petiole and the water content, and these are discussed later. The diurnal changes in total dry weight of the different parts of the plant may be assumed to be negligible, when compared with the sampling errors of the dry weight determinations.

2. Observations made on the samples.

The tops were cut off from the roots at the level of the lowest leaf. It is important to notice, therefore, that the fraction of the plant referred to subsequently as petiole also includes part of the storage organ which is morphologically stem. This tissue is proportionately much greater in sugar-beet than in mangold. The division between tops and roots was made rather higher than is usual in the agricultural operation of 'topping', where the cut is made at the widest part of the root. The detached roots were washed, dried with a cloth, and the twenty roots of a sample were weighed together.

The number of leaves on each plant was counted, leaves of the main stem and of axillary buds being recorded separately. Only leaves of length greater than 1 in. were counted, and there was no difficulty in applying this arbitrary limit for usually it fell clearly between two successive leaves, and at most there was doubt about the inclusion or non-inclusion of only one leaf. Dead leaves or part of leaves were discarded.

Ten plants were taken at random from the twenty plants of each sample, and the lamina of one leaf, selected at random from each of the ten plants, was cut off. The ten leaves were weighed separately, and their leaf areas were measured by printing on 'blue-print' paper and cutting out and weighing the prints. The leaf area of the whole sample of twenty plants was calculated, by a method previously described (Watson, 1937), from the mean fresh weight per leaf lamina obtained from the whole sample, and the mean leaf area: leaf fresh weight ratio and its regression on leaf fresh weight, calculated from the sub-sample of ten leaves.

Next, the leaf lamina of every leaf was cut off at the point of divergence of the lowest veins of the lamina from the midrib. The fresh weight of leaf lamina and of petiole of each sample of twenty plants was determined. It was found that if care was taken to avoid contact between roots and leaves during transport from the field to the laboratory, there was no need to wash the leaves. When the leaves were sampled during rain, as much surface moisture as possible was removed by vigorous shaking.

Subsamples were taken of lamina, petiole, and root for dry matter determination and carbohydrate estimation. When the plants were small, the detached leaf laminae were thoroughly mixed and the whole divided into two parts, one for dry matter and the other for carbohydrate estimation. Later (fourth sampling onwards), the leaf laminae were spread out at random in a layer 2–3 in. deep on a wooden tray, and discs were punched out with a sharp 1-in. cork-borer. Samples of about 100 gm. weight of these discs were taken. The random distribution of leaves in the tray ensured that all parts of the leaf including the edges and the veins had an equal chance of being sampled.

The roots were subsampled by cutting from each a wedge-shaped slice of a size sufficient to give a total weight of approximately 100 gm. per twenty roots. When the plants were small, they were merely halved; as they grew larger, successively smaller fractions were taken until finally only a very thin slice was necessary. The fraction required was roughly calculated from a knowledge of the total weight of root in the sample. It is known (Schubert, 1906) that the composition of the sugar-beet root varies not only longitudinally but also radially, so that a segment of the root contained between two longitudinal cuts, each of which passes through the organic centre, should give an unbiased sample of the whole root.

The petioles were subsampled in a similar way to the roots, by cutting out a segment of the crown or stem tissue with the petioles attached to it from each of the twenty plants of the sample.

The samples for dry matter estimation were dried at 100° C. in a large electric drying oven. The oven was fitted with a fan which enabled a blast of hot air to be blown over the samples. This gave very rapid drying, and preliminary drying at a lower temperature was unnecessary.

From these weights and measurements the following primary data were calculated:

- (a) Dry weight of lamina, petiole, and root.
- (b) Water content of lamina, petiole, and root.
- (c) Mean leaf area per plant.
- (d) Mean number of living leaves per plant.

The treatment of the samples taken for carbohydrate estimation will be discussed in a later paper.

3. *Measurements of rates of leaf production and of leaf death.*

Counts of the number of living leaves on the successive samples of twenty plants gave only a measure of the balance of the rates of production and death of the leaves. In order to measure the production and death rates separately, it was necessary to use the same sample of plants throughout, marking the leaves in such a way that the position of any leaf in the age series could be identified at any time.

Immediately after thinning, a sample of twenty plants was selected from each plot, and the fifth leaf was marked on the lamina with indian ink. These

plants were examined at weekly (later fortnightly) intervals, and as new leaves were produced, every fifth leaf was marked with its number. In this way the total number of leaves produced by a plant at any time could be counted, and from the difference between the total number of leaves produced and the number of living leaves present the number of dead leaves was obtained. Leaves still attached to the plant were counted as dead when the lamina was completely yellowed. This method was applied only to the leaves of the main axis. Leaves produced from axillary buds were counted but not marked, so that the rates of production and death of these leaves were not measured.

Plants which showed signs of producing flowering shoots were rejected in the selection of samples. The number of bolters was very small (16 in sugar-beet and 4 in mangold of sowing I, and 1 each in sowing II of sugar-beet and mangold and sowing V of mangold, out of approximately 800 plants per plot).

RESULTS

1. *Method of statistical analysis.*

The first complete sampling of all sowings was that of July 24-5 (sampling time 5), and to simplify the statistical analysis the earlier incomplete samplings were omitted. The remaining results form a 12×10 table for each variate, 12 treatments $\times$ 10 sampling times. The twelve treatments of the experiment were not replicated, and the estimates of error are derived from deviations from linear regressions on sowing date. There are clearly three different estimates of error: (a) involving differences between blocks, appropriate for testing the linear regression on sowing date of the mean of sugar-beet and mangold for all sampling times; (b) involving differences between plots within blocks, appropriate to the mean difference between sugar-beet and mangold for all sampling times and its regression on sowing date; (c) independent of plot differences, appropriate for testing the time interactions.

As an example of the method used, the Analysis of Variance of dry weight of petiole is given on p. 444, where S and M represent sugar-beet and mangold respectively, and S.D. sowing date.

A rearrangement of the 'Within plots between sampling times' fraction of the analysis enables the time changes of the mean of all sowing dates and of the linear regression on sowing date to be tested separately for sugar-beet and mangold. This was necessary for those growth functions, such as petiole dry weight, for which the difference between sugar-beet and mangold was large, for in such cases the error variances for sugar-beet and mangold sometimes differed significantly.

Second order regressions on sowing date were tested for those growth functions for which the data appeared to show departures from linearity in the relation to sowing date. The second order terms rarely approached significance, and with the exceptions which are noted, the linear regressions provide all the information which may legitimately be drawn from the data.

The primary data are too bulky to be published in full. All the information

Analysis of Variance

Dry weight of petiole. gm. per 20 plants.

		D.F.	Mean square.
Between blocks	Mean linear regression of S+M on S.D.	1	18265
	Deviations (a)	4	13795
Between plots with- in blocks	Mean difference, S-M	1	4625221
	Mean linear regression of S-M on S.D.	1	8855
	Deviations (b)	4	5893
Within plots between sampling times	Sampling times (Mean of S and M)	9	372741
	Interaction. Sampling times $\times$ S v. M	9	235829
	" " " $\times$ lin. regr. of S+M on S.D.	9	79596
	" " " $\times$ lin. regr. of S-M on S.D.	9	22401
	Deviations (c)	72	3538
	Total	119	

Analysis of Variance. Between Times within Plots

Dry weight of petiole. gm. per 20 plants.

	D.F.	Mean square.
Sampling times, S.	9	576319
Sampling times, M.	9	32251
Interaction of sampling time $\times$ lin. regr. of S on S.D.	9	83185
Interaction of sampling time $\times$ lin. regr. of M on S.D.	9	18812
Deviations, S.	36	6003
Deviations, M.	36	1074
	108	

derived from them, relevant to time changes and the effect of sowing date, are presented in the form of graphs (Figs. 1-14) in which the mean values for all sowings of both sugar-beet and mangold, and the linear regression coefficients on sowing date are plotted against sampling time. The standard error of the means for comparisons between sampling times is given by

$$\pm \sqrt{\frac{\text{Deviations (c) variance}}{6}},$$

and a significant difference between any two means by $3.15 \times \text{S.E.}$ ($t = 2.228$ when $n = 10$, $p = 0.05$; $\sqrt{2t} = 3.15$). Significant differences are indicated in each figure by short vertical lines.

In calculating the regression coefficients the sowing date was expressed in weeks from the first sowing. The linear regression coefficient therefore represents the increase in the growth function produced by sowing one week later. The standard error of the regression coefficients is given by

$$\pm \sqrt{\frac{\text{Deviations (c) variance}}{70}},$$

and a significant difference between any two coefficients by $3.26 \times \text{S.E.}$ ($t = 2.306$ when $n = 8$, $p = 0.05$; $\sqrt{2t} = 3.26$).

In the account of the results which follows, frequent repetition of the words 'significant' and 'not significant' has been avoided as far as possible by ensuring that definite statements are made only about effects the significance of which has been established by the Analysis of Variance. These can readily be checked by use of the 'significant differences' plotted on the graphs. Where reference is made to apparent differences of doubtful significance, a qualifying phrase to this effect has been introduced.

In the method of analysis described above, comparison is made of different sowings at the same sampling time. For some growth functions the sowings have also been compared on a scale of time reckoned from sowing date. The rearrangement of the data required for this method of comparison is easy, as both the interval between sowing dates and that between sampling times was a fortnight. The form of Analysis of Variance used is the same as that described above. The regression coefficients calculated by this method measure the effect of variation of sowing date at a given interval of time after sowing, instead of at a given calendar date.

2. Changes in dry weight.

(a) *Total dry weight.* The mean growth curves of total dry weight (Fig. 1) are of the typical S-shaped form. The initial phase of approximately exponential increase is obscured in the mean curves for sugar-beet and mangold by the omission of the first observations on the earliest sowings, but can be seen in the data for individual sowing dates. The mean dry weight for sugar-beet was consistently greater than that for mangold, but the difference did not become marked until the tenth sampling (October 2). There is some indication that the phase of rapid increase continued longer in sugar-beet than in mangold, but the trend of the curves suggests that at the last sampling time the dry weight was still increasing very slowly in both.

The linear regression coefficients of total dry weight on sowing date (Fig. 2) were consistently negative, and did not differ greatly for sugar-beet and mangold. The changes in the regression coefficient with time were small and somewhat irregular. In the early stages it fell to greater negative values at the seventh and eighth sampling, and this was followed by a steady rise, which continued to the end of the experiment. The observed variation is similar to, but smaller than, that which would be obtained if the growth curves of the different sowing dates were identical and merely displaced by successive fortnightly intervals along the time axis. That the growth curves were not, in fact, identical was shown by the regression coefficients obtained when the sowings were compared on a scale of time relative to sowing date, that is, at equal ages. This treatment of the data gave positive regression coefficients over the greater part of the period of observation. From a small initial value the coefficient rose to a maximum at about seventeen weeks after sowing, so that in this period the later sowings grew more rapidly than the early sowings. After this time the coefficient steadily decreased, becoming negative at the

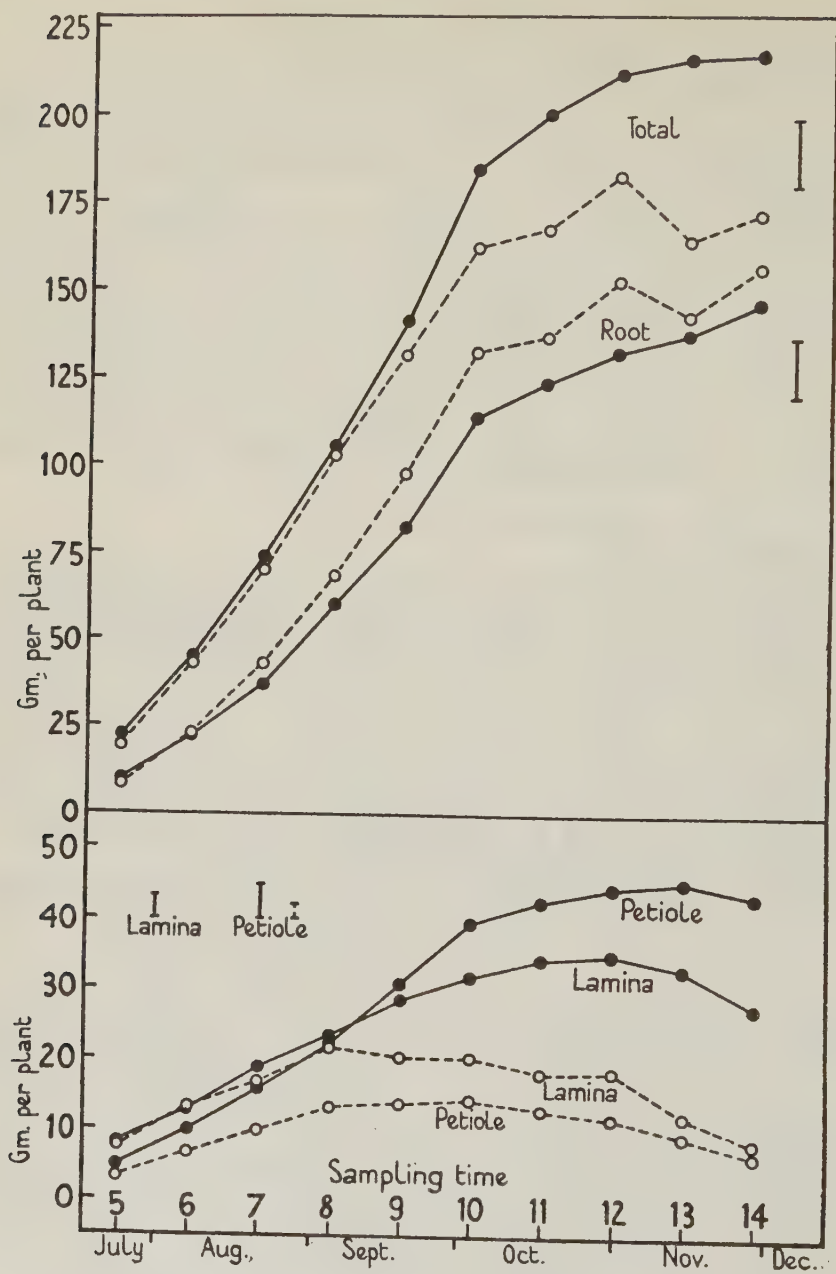


FIG. 1. Dry weight. Mean of all sowings. Full line, sugar-beet; broken line, mangold. Significant differences shown as vertical lines.

last available observation time. In this later phase, growth was less rapid the later the sowing.

The primary data show a departure from linearity in the relation of total dry weight to sowing date, in the sense that the decrease from the first to the

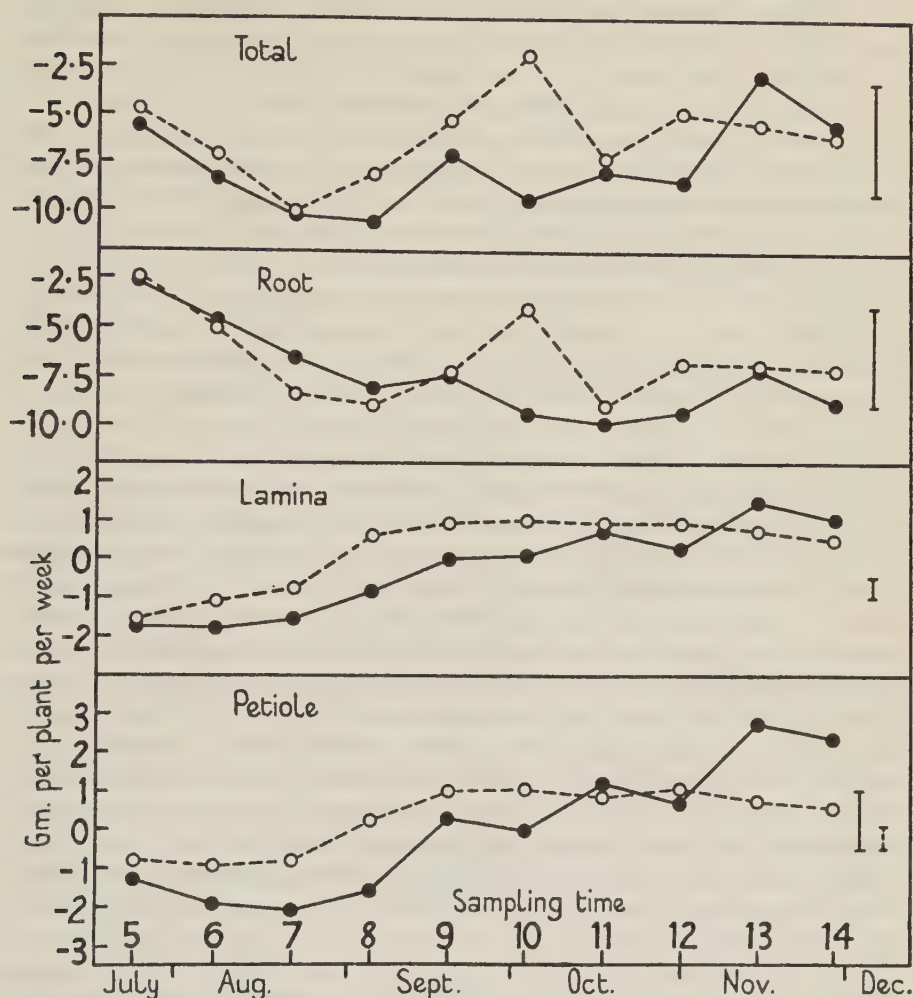


FIG. 2. Linear regression coefficients of dry weight on sowing date. Full line, sugar-beet; broken line, mangold. Significant differences shown as vertical lines.

third sowing was less rapid than that from the third to the sixth, and at some sampling times the maximum dry weight occurred at the third sowing date. This curvature was most obvious in the later samplings. When quadratic regression equations were fitted to the data, the second order regression coefficient of the mean of sugar-beet and mangold showed a significant variation with time, but was not significant for the mean of all times. The same type of curvature

was found also when the six sowings were compared on a time scale relative to sowing date.

(b) *Dry weight of leaf lamina.* The curves of mean lamina dry weight for sugar-beet and mangold (Fig. 1) were very different. For sugar-beet the mean lamina weight continued to increase until sampling 12 (October 30). For mangold the maximum was reached at sampling 8 (September 4) and after this time there was a steady fall. By the end of November the lamina weight was very small and about equal to that recorded at sampling 5 (July 24).

The linear regression coefficient on sowing date (Fig. 2) was at first negative, but rose to positive values in the later stages. Sugar-beet and mangold differed in that positive values were attained earlier in mangold, and while the values for mangold varied only slightly between sampling 9 and sampling 14, sugar-beet showed a steady rise over this period.

There is clearly no possibility of identical growth curves for the six sowings, for the later sowings ultimately produced a greater lamina weight than the early sowings. A comparison of the sowings on a time scale relative to sowing date serves only to emphasize the greater lamina weight produced by the later sowings.

(c) *Dry weight of petiole.* The changes in the petiole dry weight (Fig. 1) were similar to those observed in the dry weight of leaf lamina, but the differences between sugar-beet and mangold were much greater. The weight of petiole in the mangold was consistently greater than the lamina weight, but in sugar-beet the reverse was true, except in the early stages, when the difference between lamina and petiole weights was small. It has been pointed out that the part of the plant described as petiole included also some tissue which is morphologically stem. This tissue is much wider at its base in sugar-beet than in mangold, for the mangold has a constricted 'neck' which is not found in sugar-beet. The larger bulk of stem tissue in sugar-beet undoubtedly accounts in part for the greater weight of material recorded as petiole in sugar-beet than in mangold, but not for the whole difference, for it was obvious from inspection of the plants that the true petioles of sugar-beet were longer and larger in cross section than those of mangold. The petiole, like the lamina, showed a prolonged phase of decreasing weight in mangold and a continued rise in sugar-beet.

The linear regression coefficients on sowing date (Fig. 2) were similar in magnitude to, and showed the same time trend as, those of lamina weight. The later sowings ultimately produced a greater petiole weight than the early sowings.

A striking difference between the sowings was observed in the effects of severe frosts during the period October 30 to November 14. Many of the largest petioles of both sugar-beet and mangold of sowing VI, and to a less extent of sowing V, developed deep longitudinal splits, and were so severely damaged that they were bent over by the weight of the lamina. The smaller petioles of the earlier sowings were unaffected.

(d) *Dry weight of root.* The changes in dry weight of the root are shown in Figs. 1 and 2. No significant differences between sugar-beet and mangold could be detected, but the mean of all sowings for mangold was greater than that for sugar-beet on every sampling occasion except the first, and the difference between the means for all sampling times approached the level of significance. The trend of the curves indicates that the dry weight of the root was still increasing at the last sampling time.

The weight of the root was depressed by later sowings at all sampling times. The linear regression coefficient (Fig. 2) showed a fall to greater negative values between sampling times 5 and 8, but later it remained almost steady until the end of the experiment. Curvature in the relation to sowing date, similar to that found in total dry weight, was obvious at the later sampling times. The changes in total dry weight were dominated by the changes in dry weight of the root, for the root accounts for the major part of the weight of the plant.

(e) *Summary of dry weight changes.* The total dry weight of sugar-beet was greater than that of mangold, particularly so at the end of the sampling period, because of the greater weight of leaf lamina and petiole in sugar-beet. The dry weight of the root was smaller in sugar-beet than in mangold but the difference was not quite significant. The growth curves of the two plants were on the whole very similar. The most striking difference was found in the lamina and petioles. The dry weight of leaf lamina and petiole continued to increase over a longer period of time in sugar-beet than in mangold. The mangold showed a prolonged phase during which the dry weight of the tops steadily decreased.

By later sowing, the dry weight of the root and of the whole plant was decreased at all times to approximately the same extent in both sugar-beet and mangold. The dry weights of lamina and petiole in the early samples were also depressed by later sowing, but at a later stage this effect was reversed so that the later sowings ultimately produced a markedly greater weight of leaf than the early sowings. The change from a negative to a positive effect of sowing date took place earlier in mangold than in sugar-beet, but the positive values of the regression coefficient ultimately attained were not so great. This result of varying the date of sowing was unexpected, and is of considerable interest.

The shape of the growth curves was not independent of sowing date, for if the growth curves of successive sowings are compared on a scale of time relative to sowing date, they cannot be superimposed. The growth attained at a given interval of time after sowing was dependent not only on the length of that interval but also on environmental conditions.

3. *Changes in water content.*

The change with time of the mean water content of all sowings of sugar-beet and mangold for lamina, petiole, and root expressed as gm. per 100 gm.

dry matter are shown in Fig. 3, and the linear regression coefficients of the water content on sowing date in Fig. 4.

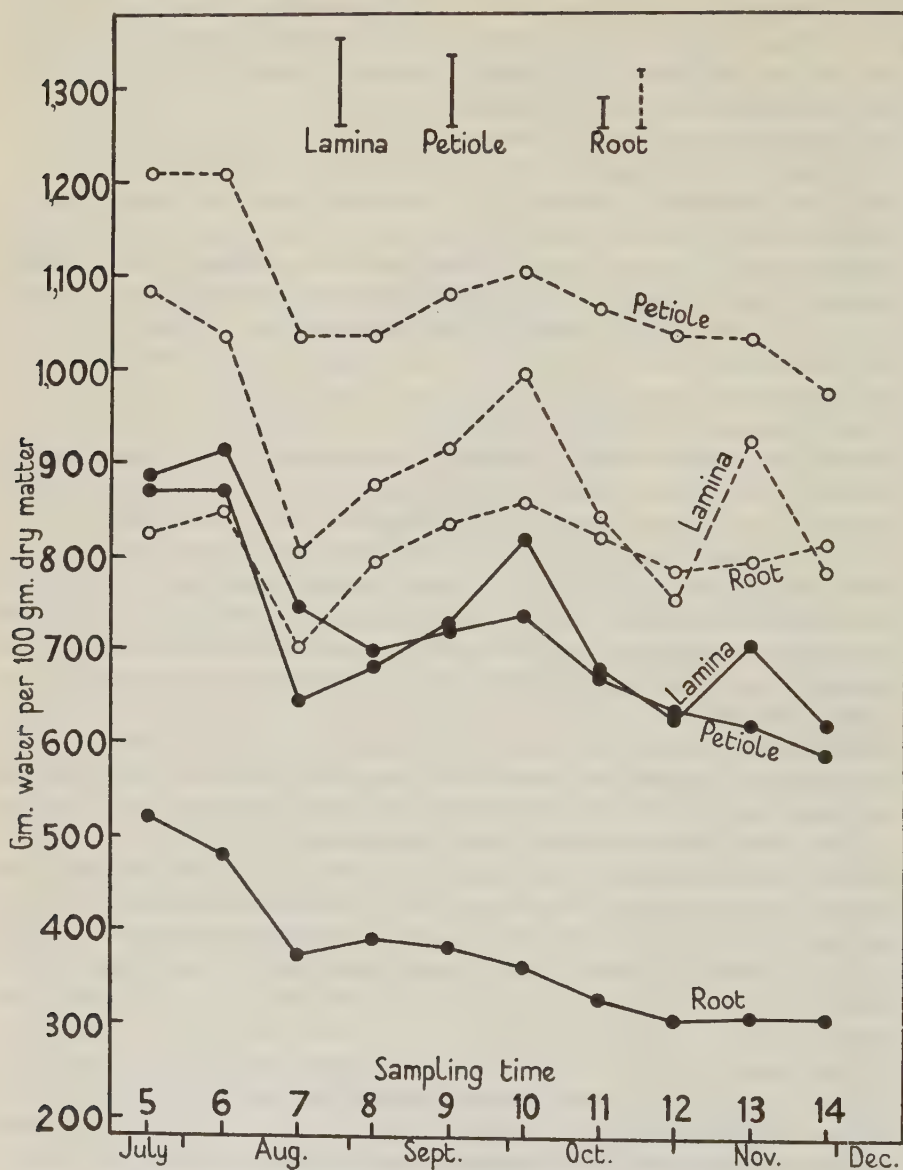


FIG. 3. Water content. Mean of all sowings. Full line, sugar-beet; broken line, mangold. Significant differences shown as vertical lines.

The water content was greater in all parts of the plant in mangold than in sugar-beet, and the largest difference was found in the root, where it was approximately twice as great in mangold as in sugar-beet. In sugar-beet the

values for lamina and petiole differed little, and were both very much greater than those of the root. In mangold the water content of the lamina was only

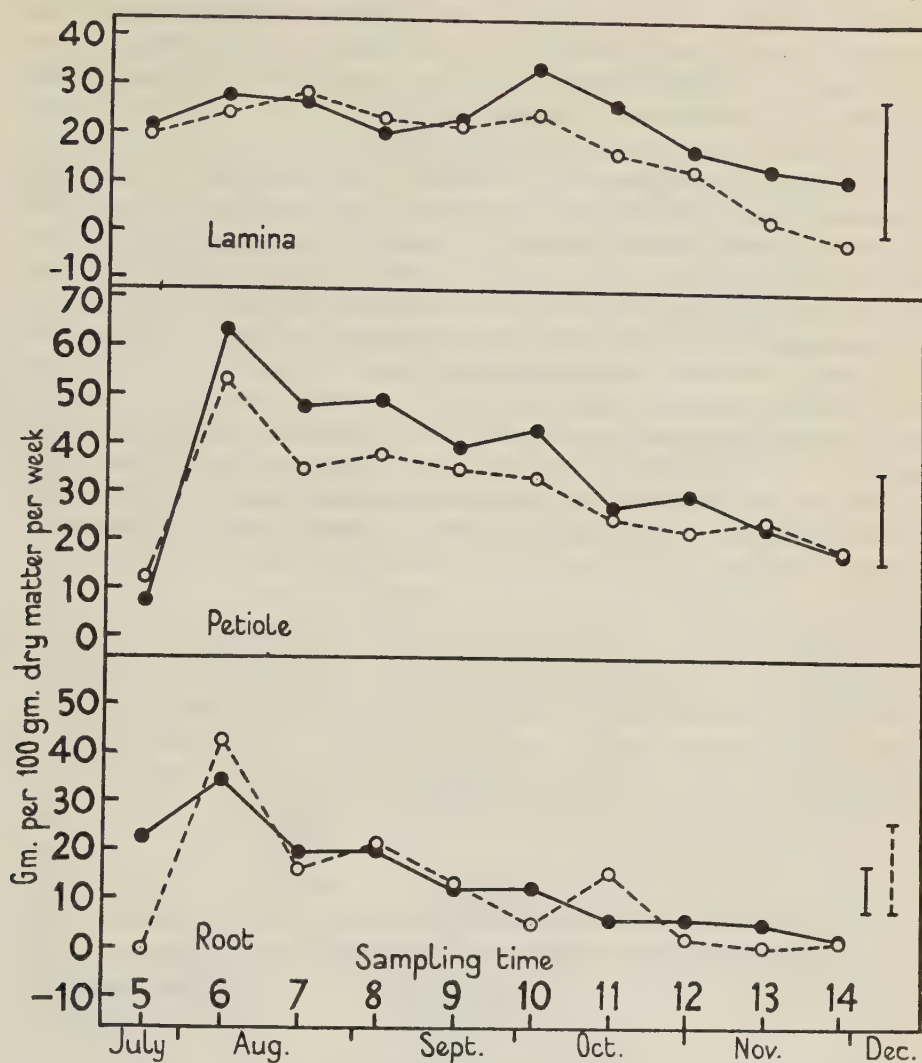


FIG. 4. Linear regression coefficients of water content on sowing date. Full line, sugar-beet; broken line, mangold. Significant differences shown as vertical lines.

slightly higher than that of the root, and the petiole had a greater water content than either lamina or root.

The mean water content, except that for mangold root, showed a downward trend over the whole growth period. The deviations from the smooth trends of all curves are obviously correlated, but they were much greater in the lamina than in petiole and root.

The linear regression coefficients of water content on sowing date (Fig. 4) were positive for all parts of the plant, and from sampling time 6 showed a downward drift with time. When the sowings were compared on a time scale relative to sowing date, the regression coefficients were still positive, so that the water content of all parts of the plant was increased by later sowing, whether the comparison between sowings was made on a scale of calendar time or on a time scale relative to sowing date.

(a) *Variation during the day.* In addition to the effect of sowing date, the variation in water content between sowings at each sampling time may have been due partly to diurnal variation, for the sowings were sampled at different times of day. There is good reason to expect large diurnal variation in the lamina, for the leaves frequently showed signs of wilting in the afternoon. The magnitude of this diurnal effect was estimated by fitting a double regression equation on time of sowing, and time of day of sampling to the values for each sampling occasion. Of the three sowings sampled on each day, the first was taken at about 10.0 a.m., the second at about 12.30 p.m., and the third at about 4.0 p.m. There was some variation in the times, but to simplify the calculation, the regression was fitted on the order of sampling instead of the actual time. On this scale the second sampling is taken as 0, the first as -1 and the third as $+1$. The regression equation fitted was of the form

$$(y - \bar{y}) = b_1(x_1 - \bar{x}_1) + b_2(x_2 - \bar{x}_2),$$

where y is the water content, x_1 the sowing date, and x_2 the time of day of sampling, expressed as the order of sampling. The regression coefficients on time of day of sampling did not differ significantly for sugar-beet and mangold, nor did they show significant variation between sampling occasions. It is sufficient, therefore, to consider the mean regressions of sugar-beet and mangold and of all occasions. These are shown in Table II.

TABLE II

*Regression of Water Content on Sowing Date and Time of Day of Sampling.
Mean of Sugar-beet and Mangold, and of all Sampling Occasions.*

			Linear regression coefficient on sowing date (b_1).	Linear regression coefficient on time of day of sampling (b_2)
Lamina	.	.	18.75 ± 1.53	-45.46 ± 6.39
Petiole	.	.	32.15 ± 1.81	-10.44 ± 7.58
Root	.	.	13.00 ± 1.19	-0.64 ± 4.97

The sowing date regressions were all significant, as has already been shown when this term was fitted alone. The mean regression on time of day of sampling was significant for the lamina; there is some indication of an effect for the petiole, but no trace for the root. The water content of the lamina decreased markedly throughout the day; that of the petiole showed a similar but smaller tendency, but in the root there was no evidence of variation during

the day. The average loss of water from the lamina between the first and third samplings during the day (10.0 a.m. to 4.0 p.m.) amounted to 11 per cent. of the mean water content.

The factors of the environment which may induce diurnal variation of water content, namely temperature, humidity of the external atmosphere, and humidity of the internal atmosphere of the leaf, which is subject to stomatal control, act at the lamina end of the gradient of suction pressure which determines water movement through the plant. At the other end of the gradient, the suction pressure of the soil is unlikely to vary appreciably during the course of a day. Diurnal fluctuations of transpiration rate should therefore be associated with progressively smaller changes of suction pressure, and hence of water content, in the direction from lamina to root. The changes in the root would be expected to be very small as it is embedded in a medium whose suction pressure is almost constant throughout the day.

The root is mechanically a more rigid structure than the leaf, and for this reason it is probable that a given change of suction pressure is associated with a smaller change of water content in the root than in the leaf.

These two factors together furnish an explanation of the observed differences between lamina, petiole, and root in the diurnal variation of water content.

(b) *Variation between sampling times.* It has been pointed out that there is an obvious correlation between the deviations from a smooth trend of the mean water content of lamina, petiole, and root, suggesting that the deviations were not due to experimental errors but to the influence of an external factor.

The factor most likely to be the cause of these variations is the water content of the soil. No direct measurements of soil water content at the time of sampling are available, but an indirect measure can be used. The water content of the soil is determined by the accumulated rainfall, less the amount of water lost by evaporation from the soil surface, by drainage, and by transpiration through the plant, so that we may use accumulated rainfall less a function of time, which represents the water loss, as an indirect measure of soil water content. Assuming, as a first approximation, a constant rate of water loss from the soil, the method adopted was to fit a regression equation of the form

$$(y-\bar{y}) = b_1(x-\bar{x}) + b_2(t-\bar{t}),$$

where y is the mean water content of lamina, petiole, or root, x is the accumulated rainfall, and t is time.

The falling trend of water content of the plant throughout the experimental period may have been due either to a steady fall of soil water content or to internal factors of the plant independent of water supply, or to both. The time term of the regression takes account of both effects and it is not possible to separate them. If it can be assumed, however, that the time drift of water content of the plant was not influenced by internal factors but was determined

entirely by the water content of the soil, the regression equation may be written as

$$(y - \bar{y}) = b_1[(x - \bar{x}) + \frac{b_2}{b_1}(t - \bar{t})]$$

and the function $[(x - \bar{x}) + \frac{b_2}{b_1}(t - \bar{t})]$ becomes a measure of the deviation at time t of the soil water content from its mean; $-\frac{b_2}{b_1}$ represents the mean rate of water loss from the soil, and b_1 is the change in water content of the plant tissue corresponding to unit change in soil water content.

The values of the regression coefficients and their standard errors, calculated from the mean water content of all sowings for sugar-beet and mangold for the period from sampling time 5 to 14, are shown in Table III.

TABLE III

Regression Coefficients of Water Content (Mean of all Sowing Dates) on Accumulated Rainfall in Inches (b_1) and Time in Weeks (b_2).

		Sugar-beet.	Mangold.	Mean of sugar-beet and mangold.
Lamina.	b_1	<i>124.4 ± 44.2</i>	<i>163.6 ± 45.6</i>	<i>144.0 ± 43.9</i>
	b_2	<i>-76.1 ± 23.6</i>	<i>-97.9 ± 24.3</i>	<i>-87.0 ± 23.5</i>
	b_2/b_1	<i>-0.612</i>	<i>-0.598</i>	<i>-0.604</i>
Petiole.	b_1	<i>32.4 ± 36.7</i>	<i>76.7 ± 40.8</i>	<i>54.6 ± 38.2</i>
	b_2	<i>-33.3 ± 19.6</i>	<i>-49.7 ± 21.8</i>	<i>-41.5 ± 20.4</i>
	b_2/b_1	<i>-1.027</i>	<i>-0.648</i>	<i>-0.760</i>
Root.	b_1	<i>28.9 ± 24.6</i>	<i>73.5 ± 28.6</i>	<i>51.2 ± 23.9</i>
	b_2	<i>-26.3 ± 13.2</i>	<i>-38.7 ± 15.3</i>	<i>-32.5 ± 12.7</i>
	b_2/b_1	<i>-0.910</i>	<i>-0.526</i>	<i>-0.635</i>

Significant results in italics.

The double regression was highly significant in all cases, reducing the error variance for the lamina by 70 per cent., for the petiole by 71 per cent., and for the root by 59 per cent. The rainfall and time terms considered separately were significant for the lamina and for mangold root, but not for the petiole or for sugar-beet root. The regression coefficients were consistently greater for mangold than for sugar-beet, significantly so for petiole and root. The results are in agreement with the hypothesis that the variation in water content of the plant was caused by variation in water content of the soil, for it is difficult to suggest any reason for the correlation with accumulated rainfall other than its effect in controlling the soil water content.

An illustration of the goodness of fit of the regression equation is given in Table IV, where the observed and calculated values of mean water content of the lamina are set out.

The difference between the calculated and observed values is negative at the beginning and end of the period and generally positive in the middle region, suggesting that the rate of water loss from the soil was not constant, but was higher in the middle of the period than at the beginning and end.

The ratio of the regression coefficients b_2/b_1 (Table III), is fairly constant, as would be expected if it were a real measure of the rate of water loss from the soil. The values for sugar-beet petiole and root are somewhat higher than the others, but here the regression coefficients are smaller and have proportionately greater errors, so that the ratio is less accurately determined. In the

TABLE IV

Water Content of Lamina. gm. per 100 gm. Dry Matter. Mean of Sugar-beet and Mangold, and of all Sowing Dates.

Sampling time.	Accumulated rainfall in inches.	Observed.	Calculated from regression equation.	Calculated — observed.
5	0	976.5	963.2	—13.2
6	0.520	951.7	864.1	—87.6
7	0.951	724.0	752.2	28.2
8	2.864	780.1	853.8	73.7
9	3.961	822.2	837.7	15.5
10	5.483	906.0	882.9	—23.1
11	6.233	761.4	817.0	55.6
12	6.752	691.4	717.7	26.3
13	8.470	816.2	791.2	—25.0
14	8.707	701.8	651.3	—50.5

absence of any information as to the drift of water content of the plant with time due to internal factors, the mean value of the ratio, -0.67 , may be interpreted as indicating an average rate of water loss from the soil of about 0.7 in. of rain per week.

The regression coefficients are much greater for the lamina than the petiole, and slightly greater for the petiole than the root. This result is similar to that found for variation during the day, namely, that the water content of the lamina is much more variable than that of petiole and root. It must be attributed to the greater mechanical rigidity of root and petiole, for here we are concerned with variations of suction pressure of the soil, which must be accompanied by approximately the same variations of suction pressure throughout the plant, whereas in the diurnal changes the variation is induced at the lamina end of the suction pressure gradient, the suction pressure of the soil remaining steady. This distinction accounts for the fact that while the root water content showed no variation during the day, it varied considerably between sampling times. The larger regression coefficients found for mangold than for sugar-beet indicate that the water content of mangold is more sensitive to changes of soil water content than that of sugar-beet. The explanation of this may lie in differences of mechanical structure and of osmotic relations of the tissues.

It is possible that the fitting of a multiple regression of water content of the plant on accumulated rainfall and time might be made the basis of a method for estimating the water requirement of plants growing in the field, for which no other method is available at present. It must be noted, however, that the rate of loss of water from the soil through the plant can be determined from

the ratio of the regression coefficients b_2/b_1 only if loss by drainage or direct evaporation, and any drift with time of the water content of the plant due to changes in internal factors, can be measured or eliminated.

4. *Changes in leaf number.*

(a) *Number of leaves per plant.* Two sets of observations were made on leaf number, the first derived from the plants sampled for dry weight estimation, and the second from the plants on which the leaves were marked as they were produced, so that the number of a leaf in the age series could be determined at any time. The two sets of samples will be referred to as the 'sampled plants' and the 'marked plants' respectively. They differ in that while the same marked plants were used throughout, the 'sampled plant' observations were made on different plants at each observation time. The marked plants provide a more accurate estimate of the time changes than the sampled plants, as they were not subject to plant to plant variation but, on the average of all times, the sampled plants give a more representative sample of the whole population. In this section, only leaves on the main axis will be considered.

The changes in the mean number of living leaves present per plant are shown in Fig. 5. The results for the sampled and marked plants were similar, except that the number of leaves on the marked plants was slightly greater than on the sampled. Sugar-beet had a greater number of leaves than mangold and the difference rapidly increased with time, so that at the end of the experiment sugar-beet had nearly twice as many leaves as mangold.

The linear regression coefficient of leaf number on sowing date (Fig. 5) was at first large and negative, and greater in sugar-beet than in mangold. For a short time the regression coefficient tended to rise to smaller negative values, but from the beginning of September onwards the rise ceased and there was no steady drift with time. The magnitude of the change in the regression coefficient was almost equal in sugar-beet and mangold. From September onwards the regression coefficient for mangold was almost zero so that all sowings had the same leaf number, but owing to the greater initial negative values in sugar-beet, the coefficient continued to be negative throughout the whole period, and the leaf number decreased consistently with later sowing.

(b) *Rate of leaf production.* The observations on the marked plants enable an analysis of the changes in leaf number to be made in terms of the weekly rates of production of new leaves and of death of old leaves. A peculiar feature of the sugar-beet plants caused some difficulty in making the counts in the later stages. In 20 plants out of the 120 selected, a split developed in the crown of the root, so that the apical meristem was divided and the regular phyllotaxis was interrupted. New leaves produced from the sides of the half meristems next to the split were frequently deformed. Occasionally they grew downwards into the fissure and their development was arrested. Only one mangold plant out of 120 selected showed this phenomenon. In sugar-beet it was

found in all sowings, and the largest plants were usually those affected. The splitting appeared to be mechanical in origin, for no symptoms of disease were visible, and the tissues exposed in the split developed a callus.

The mean rate of production of leaves per plant per week is given in Fig. 6. The difference between the means for sugar-beet and mangold was not quite

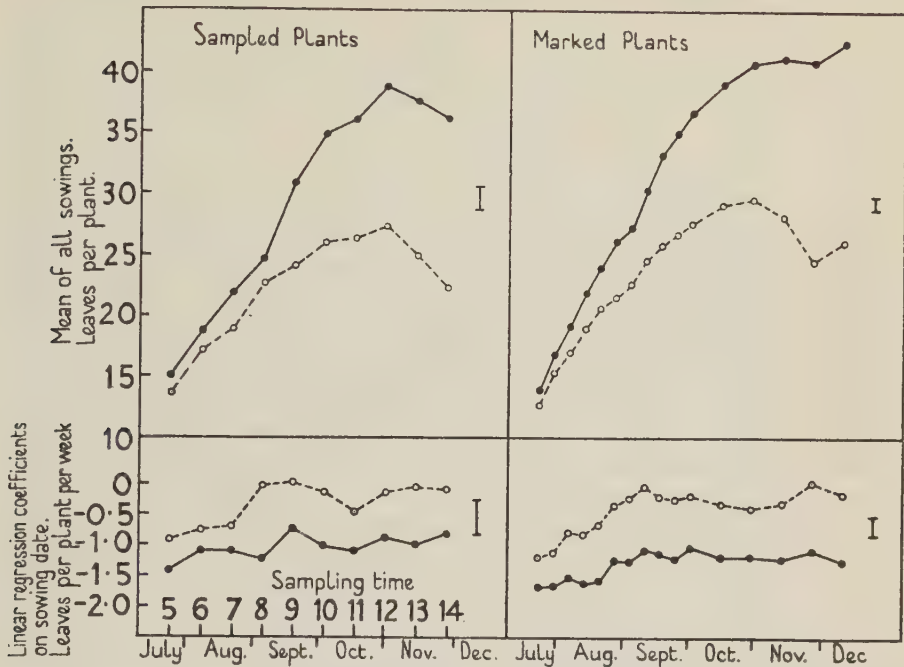


FIG. 5. Number of living leaves per plant. Full line, sugar-beet; broken line, mangold. Significant differences shown as vertical lines.

significant, but the values for sugar-beet were usually slightly greater than those for mangold. The rate tended to fall over the whole sampling period, but there were wide and significant fluctuations. The coincidence of these fluctuations in sugar-beet and mangold suggests that some meteorological factor was affecting the rate.

The effect of sowing date and its variation with sampling time were similar for sugar-beet and mangold. From a positive value at the first sampling time, the linear regression on sowing date fell to zero and later to negative values. These changes in the regression coefficient indicate that the steepness of the decline of the rate of leaf production with time was greater the later the sowing, and that the curves for the different sowings intersect at the time when the regression coefficient is zero. It is clear therefore that on a time scale relative to sowing date the curves are not identical.

The weekly mean temperature, calculated as the average of the weekly mean maximum and the weekly mean minimum temperatures, is also plotted in

Fig. 6, and there is an obvious correlation, both in trend and in deviations from the trend, between mean temperature and rate of leaf production.

Linear regressions on weekly mean temperature were fitted for each sowing

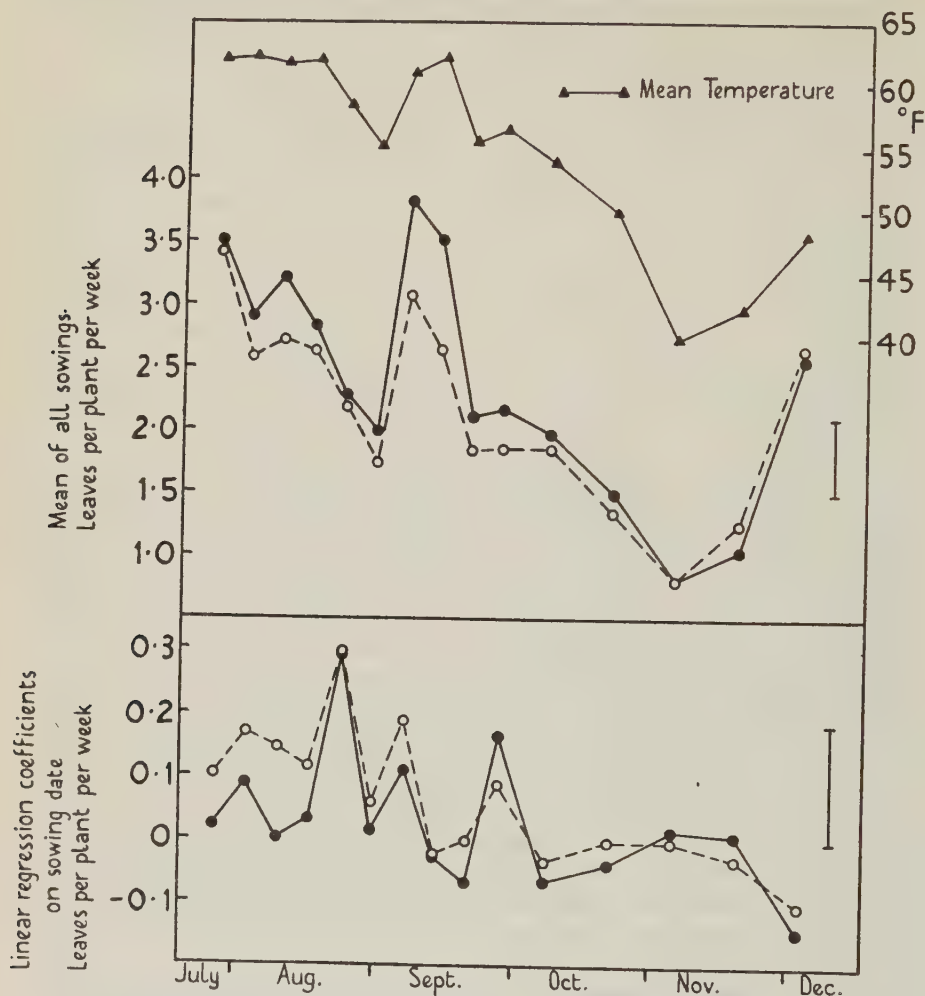


FIG. 6. Rate of leaf production. Full line, sugar-beet; broken line, mangold. Significant differences shown as vertical lines.

of sugar-beet and mangold. The regression coefficients are shown in the first line of Table V.

The mean regression was highly significant, reducing the error variance by 50 per cent., but the difference between the regressions for sugar-beet and mangold was not significant. The regression coefficients for the six sowing dates did not differ widely, but a tendency to increase with later sowing was evident, as would be expected from the fact that the rate of leaf production declined

more rapidly with time the later the sowing. The increase in the regression coefficient continued throughout the whole range of sowing dates, and was found to be significant when estimated as a linear regression on sowing date.

In view of this variation between sowings in the relation of the rate of leaf

TABLE V

Linear Regression Coefficients of Rate of Leaf Production on Weekly Mean Temperature (Mean of Sugar-beet and Mangold). Leaves per Plant per Week per °F.

Sowing date.	I.	II.	III.	IV.	V.	VI.
Trend not eliminated	0.0697	0.0819	0.0958	0.1050	0.1117	0.1242
Trend eliminated	0.0684	0.0843	0.1307	0.0987	0.0927	0.1000

production to temperature, it is of importance to consider whether the downward trend of the rate is attributable to the temperature trend, or to some other factor. Information on this point can be obtained by examining the relation between deviations, after elimination of trend from both rate of leaf production and temperature. For ease of calculation, a linear time trend was assumed. The values of the linear regression coefficients of the deviations from the time trend of the rate of leaf production on temperature are shown in the second line of Table V. The steady increase with later sowing date disappeared with the elimination of trend, and no significant difference between sowing dates in the regression coefficients of deviations from trend could be detected. Thus the relation between deviations from trend of the rate of leaf production and temperature was independent of sowing date, which would be expected if the deviations in the rate were induced by temperature variation.

The regression coefficient of the mean rate of leaf production for all sowings on temperature before elimination of trend was 0.0963 ± 0.0070 , and for the deviations from the time trend, 0.0965 ± 0.0140 . These coefficients are almost identical, and this suggests that the downward trend of the rate was causally related to the temperature trend. It seems justifiable to conclude that the variations in the rate of leaf production, both in trend and short period deviations, were determined mainly by temperature. To account for the variation between sowing dates of the regression on temperature when trend was not eliminated, the operation of an internal factor dependent on sowing date must be assumed whose effect on the rate of leaf production was to cause a more rapid fall with time the later the sowing.

It is very improbable that the relation between rate of leaf production and temperature is truly linear. Fig. 7, in which the mean rate of leaf production is plotted against temperature, suggests that the relation is of the usual van't Hoff exponential form. Assuming that this is so, the values of the temperature coefficient Q_{10} can be calculated by fitting linear regressions of the logarithms of the rates of leaf production on temperature. The regression coefficients obtained are shown in Table VI.

The coefficients are not significantly different for sugar-beet and mangold.

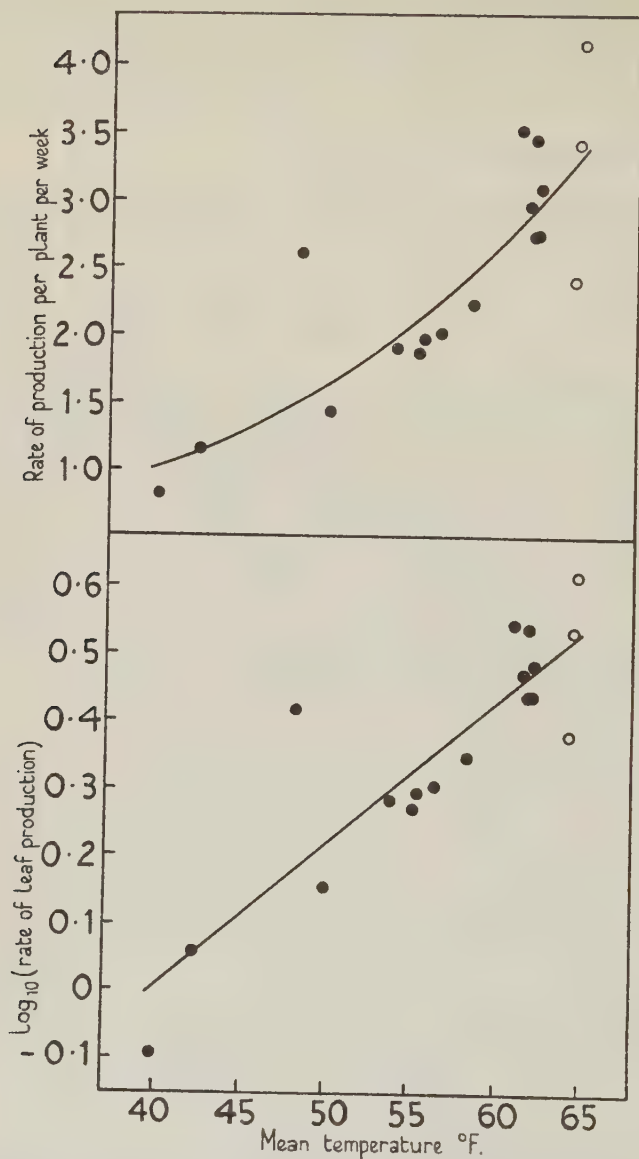


FIG. 7. Relation between the rate of leaf production (mean of all sowings of sugar-beet and mangold) and temperature. The straight line in the lower half of the figure is the calculated linear regression line of $\log_{10}$ (rate of leaf production) on mean temperature, and the curved line in the upper half of the figure is the corresponding line when rate is plotted against temperature.

The points shown by open circles are means for the first three or first four sowings only, for the period before the later sowings were thinned.

TABLE VI

Linear Regression Coefficients of $\log_{10}$ (Rate of Leaf Production) on Temperature ($^{\circ}\text{F}$). (Mean of all Sowings).

	Sugar-beet.	Mangold.	S.E.	Mean of S. and M.	S.E.
Including trend	0.02275	0.01999	± 0.002898	0.02137	± 0.002049
Deviations from trend	0.03199	0.02265	± 0.005729	0.02732	± 0.004051

nor does the elimination of trend produce a significant difference. In the lower half of Fig. 7 the logarithm of rate of leaf production is plotted against temperature, and the fitted linear regression line is shown. The values of Q_{10} may be calculated from the regression coefficients as follows:

If x_1 is the rate of leaf production at temperature $T^{\circ}\text{C}$. ($= t^{\circ}\text{F}$.) and x_2 is the rate at $T+10^{\circ}\text{C}$. ($= t+18^{\circ}\text{F}$.), then Q_{10} is defined as the ratio $\frac{x_2}{x_1}$.

The regression equation fitted to the data is of the form

$$\log_{10} x = bt + c,$$

where t = temperature in $^{\circ}\text{F}$., and the values of the regression coefficient b are given in Table VI.

$$\text{Then } \log_{10} \frac{x_2}{x_1} = b(t+18) - bt = 18b,$$

$$\frac{x_2}{x_1} = Q_{10} = 10^{18b}.$$

The value of Q_{10} before elimination of trend is 2.42, and that for deviations from trend is 3.10. These do not differ significantly since their logarithms have been shown to be not significantly different. The temperature coefficients are of the order of those found for chemical reactions and for many biological processes, which supports the conclusion that the correlation observed between temperature and the rate of leaf production is a causal and not a chance effect.

An interesting feature of the graphs in Fig. 6 is that at times when the temperature had risen after a sharp fall, the rate of leaf production was greater than would be expected from the prevailing temperature. This is obvious in the points for the period September 3-10 and particularly November 26-December 10. These points appear as positive deviations from the regression line in Fig. 7, where rate of leaf production is plotted against temperature. This suggests that low temperatures had an after-effect, causing a greater acceleration of the rate of leaf production in a subsequent period than could be attributed to rise of temperature.

The rate of leaf production, as here defined, is not purely a measure of meristematic activity; it is also a function of the rate at which preformed leaf initials develop to a length of one inch. It is difficult to determine the extent to which observed temperature relations are a measure of the effect of

temperature on the production of new leaf initials by the meristem, or on their elongation to the arbitrary size limit at which they were included in the leaf counts. Over a long period of time the rate of leaf production must be a measure of the activity of the meristem, for it is impossible for more leaves to develop than there are initials formed, and highly improbable that there should be any appreciable increase over a long period of time in the number of undeveloped leaf initials. The time trend is, therefore, probably the expression of a variation of meristematic activity, and it is of interest to note that its temperature coefficient is of the same order as that characteristic of chemical reactions.

(c) *Death rate of leaves.* The rate of death of leaves per week is plotted in Fig. 8. The death rate was nearly twice as great in mangolds as in sugar-beet. Over the period from July to the end of October the death rate showed no obvious trend, but later it increased rapidly. There is little doubt that this rise was caused by frost damage, for many leaves at this time showed the typical symptoms of flaccidity and injection and, in the later sowings, splitting of the petioles.

Apart from the final rise, the death rate appeared to vary independently for the different sowings and no correlation with any weather factor could be detected. The death rate takes account not only of those leaves which passed through the natural processes of senescence and death while attached to the plant, but also of leaves which were accidentally broken off in the course of cultivation and sampling, so that its determination was more subject to error than the rate of leaf production.

The linear regression coefficient of the death rate on sowing date showed a downward drift from positive values in the early stages to negative values, but the changes with time were small. There was no difference between sugar-beet and mangold in the effect of sowing date.

It is clear that the development of a greater number of leaves in sugar-beet than in mangold was due mainly to a greater death rate in mangold, and partly to a slightly greater rate of production in sugar-beet. The effect of sowing date on the rates of leaf production and death was the same for sugar-beet and mangold, and this accounts for the observed similarity of the change with time in the effect of sowing date on leaf number in sugar-beet and mangold. The only difference between sugar-beet and mangold in the effect of sowing date on leaf number was the greater depression with later sowing observed at the early sampling times in sugar-beet. This developed before the observations began, and was not affected by any subsequent changes in the rates of leaf production and death.

The total number of leaves produced per plant, and the number which died in the whole period of growth from sowing to 10 December are shown in Table VII.

These numbers represent the integration of production rate and death rate over the whole period, and show the resultant of the changes observed in the rates, and of the variation in the length of the growing period for the different

sowings. Both the number of leaves produced and the number dead decreased with later sowing. This was mainly the result of the shortening of the growing period and not an effect of sowing date on the production and death rates, for both production rate and death rate increased with later sowing in the early

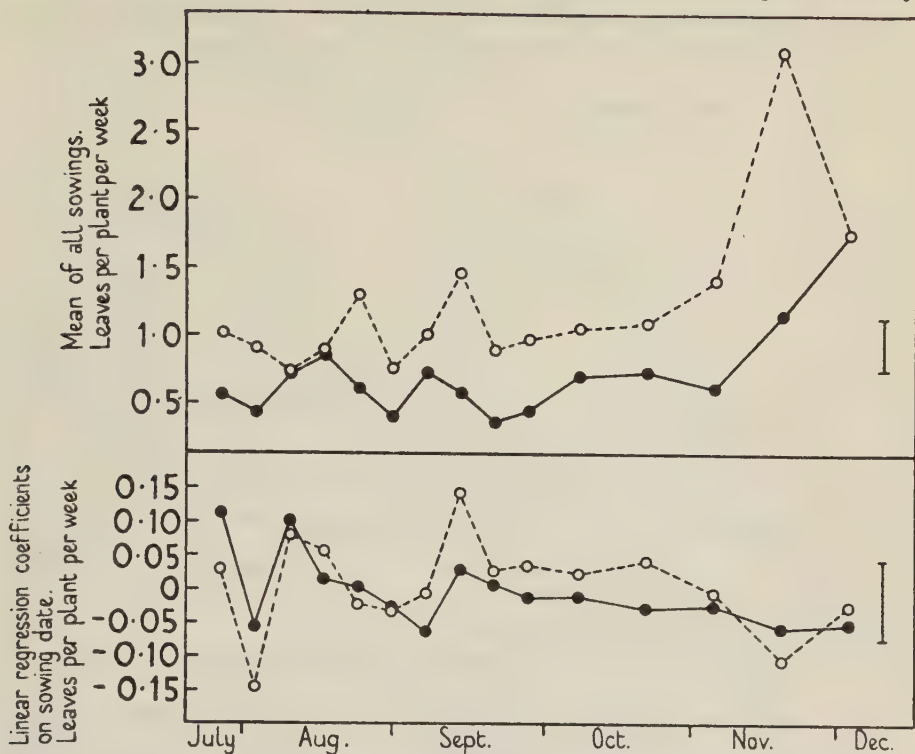


FIG. 8. Death rate of leaves. Full line, sugar-beet; broken line, mangold. Significant differences shown as vertical lines.

TABLE VII

Number of Leaves Produced per Plant, and Number which had Died, in the Period from Sowing to December 10. (S = Sugar-beet, M = Mangold).

Sowing date	I.		II.		III.		IV.		V.		VI.	
	S.	M.	S.	M.	S.	M.	S.	M.	S.	M.	S.	M.
Leaves produced	68.5	62.3	72.0	59.2	64.6	60.2	52.5	55.7	52.6	52.3	53.8	51.1
Leaves dead	22.0	35.7	21.0	33.7	19.8	32.0	15.1	29.8	15.2	27.2	15.5	25.5

stages and decreased with later sowing in the later stages, so that there was little difference between sowing dates in the mean rates over the whole period. The total number of leaves produced was slightly greater and the number dead was markedly less in sugar-beet than in mangold, in accordance with the consistent differences observed in the rates of production and death.

Casual inspection of a root crop in the field might suggest that the tops were

in a relatively static condition, and that there was little growth activity apart from a steady accumulation of storage products in the root. Table VII emphasizes that this is far from a true picture. Throughout the period of vegetative growth there was a steady loss and renewal of leaves, so that in sugar-beet about one-third and in mangold one-half of the leaves produced were lost, mainly by normal senescence but partly by accidental damage.

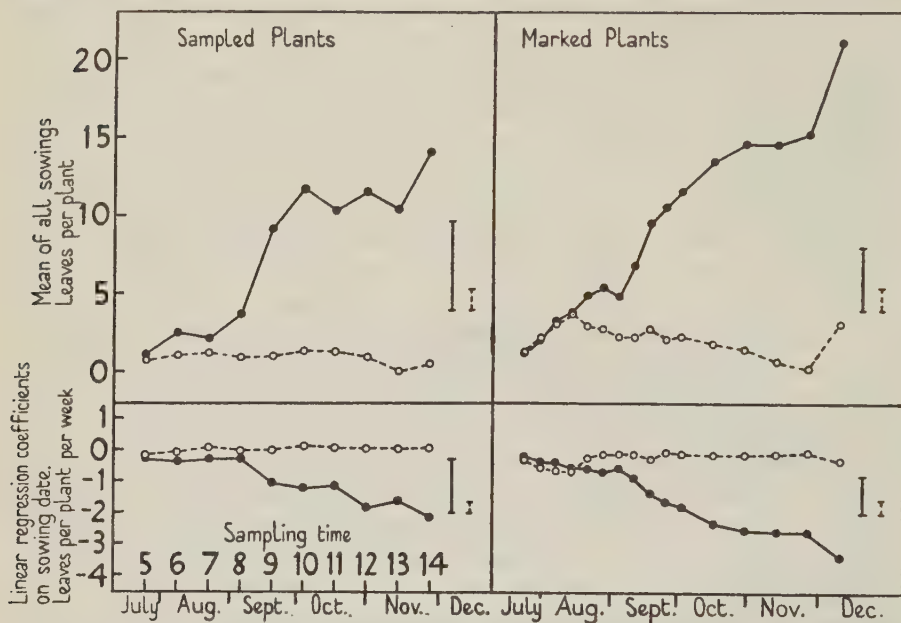


FIG. 9. Number of axillary leaves per plant. Full line, sugar-beet; broken line, mangold. Significant differences shown as vertical lines.

(d) *Number of axillary leaves per plant.* The number of axillary leaves, that is to say leaves produced from lateral buds, was counted on both sampled and marked plants, but they were not marked in order of production, so that no information on their rates of production and death is available. The results are given in Fig. 9.

The mangold plants produced very few axillary leaves, and the differences between sowing dates were negligible. In sugar-beet the number of axillary leaves per plant increased steadily with time, and at all times it was much greater for the early sowings than the late. This can be seen in Fig. 9 from the large negative linear regression coefficients on sowing date. There was some evidence of a departure from linearity in the sowing date effect, for the first sowing had fewer axillary leaves than the second and third sowings, but there was a steady decrease from the second to the sixth sowings.

(e) *Mean dry weight of lamina per leaf.* The extent to which variations in leaf number account for variations in lamina dry weight per plant can be tested by an examination of the mean lamina dry weight per leaf (Fig. 10).

The lamina dry weight per leaf was slightly greater in mangold than in sugar-beet in the early stages of growth, and this offset the smaller leaf number, so that the difference in lamina dry weight per plant was small. The lamina dry weight per leaf increased to a maximum at the same time in both

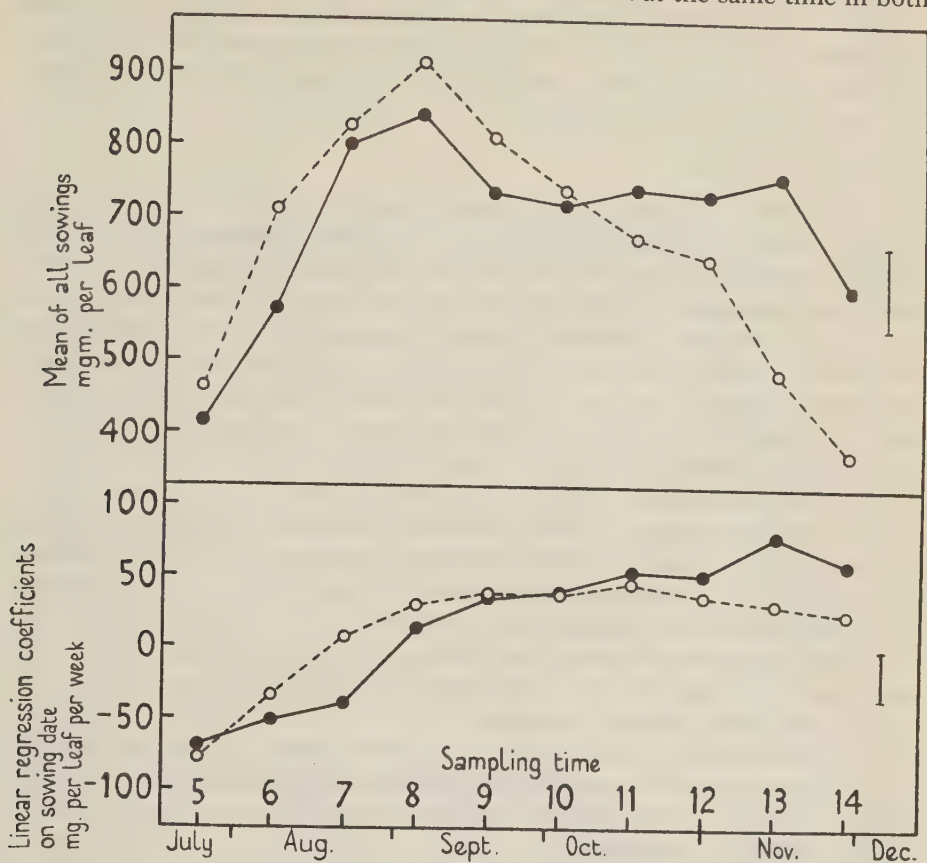


FIG. 10. Mean dry weight of lamina per leaf. Full line, sugar-beet; broken line, mangold. Significant differences shown as vertical lines.

plants, but the subsequent fall was steeper in mangold so that in the later stages the values for sugar-beet were considerably greater than those for mangold. There were many axillary leaves on the sugar-beet plants, and few in mangold, and as the axillary leaves were very small compared with those on the main axis, the difference between sugar-beet and mangold in lamina dry weight per leaf of the main stem must have been much greater than that shown in Fig. 10, where all leaves are included in the means. The large difference between sugar-beet and mangold in lamina dry weight per plant, developed in the later stages of growth, was due to the greater lamina dry weight of individual leaves reinforcing the greater leaf number in sugar-beet.

The linear regression coefficient of lamina dry weight per leaf on sowing

date (Fig. 10) rose rapidly from initial negative values, to positive values over the greater part of the growth period. The rise was steeper but continued for a shorter time in mangold than in sugar-beet, matching the similar changes observed in the regression of lamina weight per plant. Thus, the greater lamina dry weight per plant produced by later sowing was due to an increase in lamina dry weight per leaf, and this effect was augmented by a tendency for the later sowings to catch up on the early sowings in leaf number, which was particularly marked in mangold.

5. *Changes in leaf area.*

(a) *Leaf area per plant.* The changes in the area of leaf lamina per plant (Fig. 11) follow very closely those already described in the dry weight of leaf lamina. Both sugar-beet and mangold showed a steady increase in leaf area until early September, and there was little difference between them. After this time the leaf area of mangold began to decrease, while that of sugar-beet continued to increase, so that in the later stages the leaf area of sugar-beet was much greater than that of mangold. The difference between sugar-beet and mangold in leaf area was proportionately less than their difference in lamina dry weight.

The linear regression coefficient on sowing date (Fig. 12) was at first negative, but rose to positive values over the greater part of the growth period. The rise was more rapid, but less prolonged, in mangold than in sugar-beet. Thus the effect of late sowing in increasing the lamina dry weight per plant, was accompanied by an increase in leaf area.

In Fig. 11 a scale of leaf area per unit area of land occupied by the crop is also given. The leaf area per acre reached a maximum of just over 4 acres in sugar-beet and 3 acres in mangold.

(b) *Mean area of leaf lamina per leaf.* The mean area of leaf lamina per leaf (Fig. 11) was greater in mangold than in sugar-beet except at the end of the growth period, so that the greater leaf area per plant in sugar-beet is attributable to greater leaf number.

The effect of sowing date on the mean area per leaf (Fig. 12) was similar to that observed on the leaf area per plant. The increase in leaf area per plant produced by late sowing was due mainly to an increase in the area of individual leaves, for it has been shown (Figs. 5 and 9) that the number of leaves per plant was not increased at any time by later sowing.

The great difference of leaf size between the sowings in the later stages of growth was associated in sugar-beet with a striking change of the habit of the plant. Towards the end of October the leaves of many plants of the early sowings assumed a flat rosette form with short petioles, while the later sowings still had erect leaves with much longer petioles. Almost all the plants of sowings I and II had the rosette form, and there were a few in sowings III and IV, but none in the later sowings.

(c) *Leaf area per unit dry weight of lamina.* In view of the close similarity

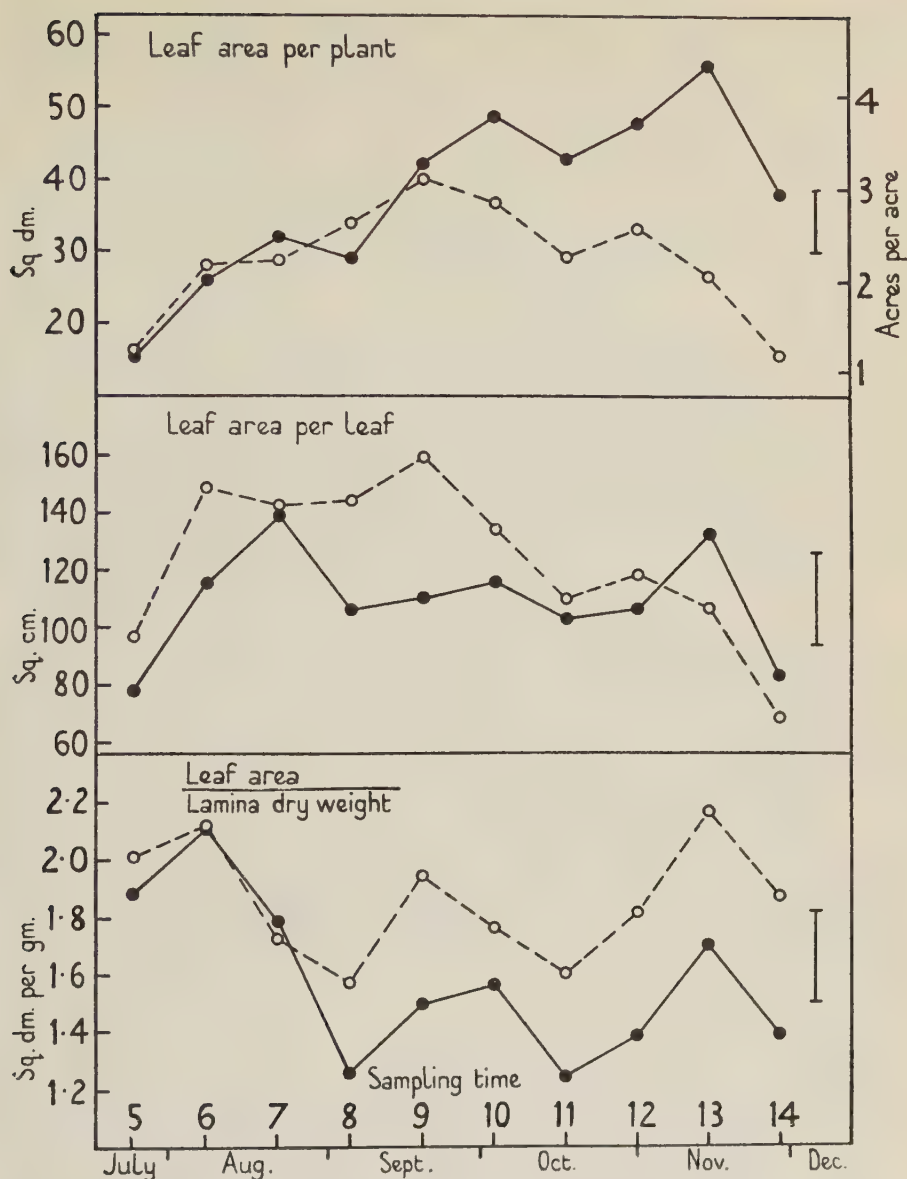


FIG. 11. Leaf area per plant, leaf size and ratio of area to lamina dry weight. Means of all sowings. Full line, sugar-beet; broken line, mangold. Significant differences shown as vertical lines.

between the curves of leaf area and lamina dry weight per plant it is important to examine the variations in the leaf area/lamina dry weight ratio. If this ratio were sensibly constant, the dry weight of leaf lamina would be an adequate measure of leaf area. It has been shown (Watson, 1937) that in leaves from a

homogeneous population of plants the leaf area/leaf weight ratio decreases with increase in leaf weight. The mean lamina dry weight per leaf has been shown to vary with time and sowing date and to differ for sugar-beet and man-

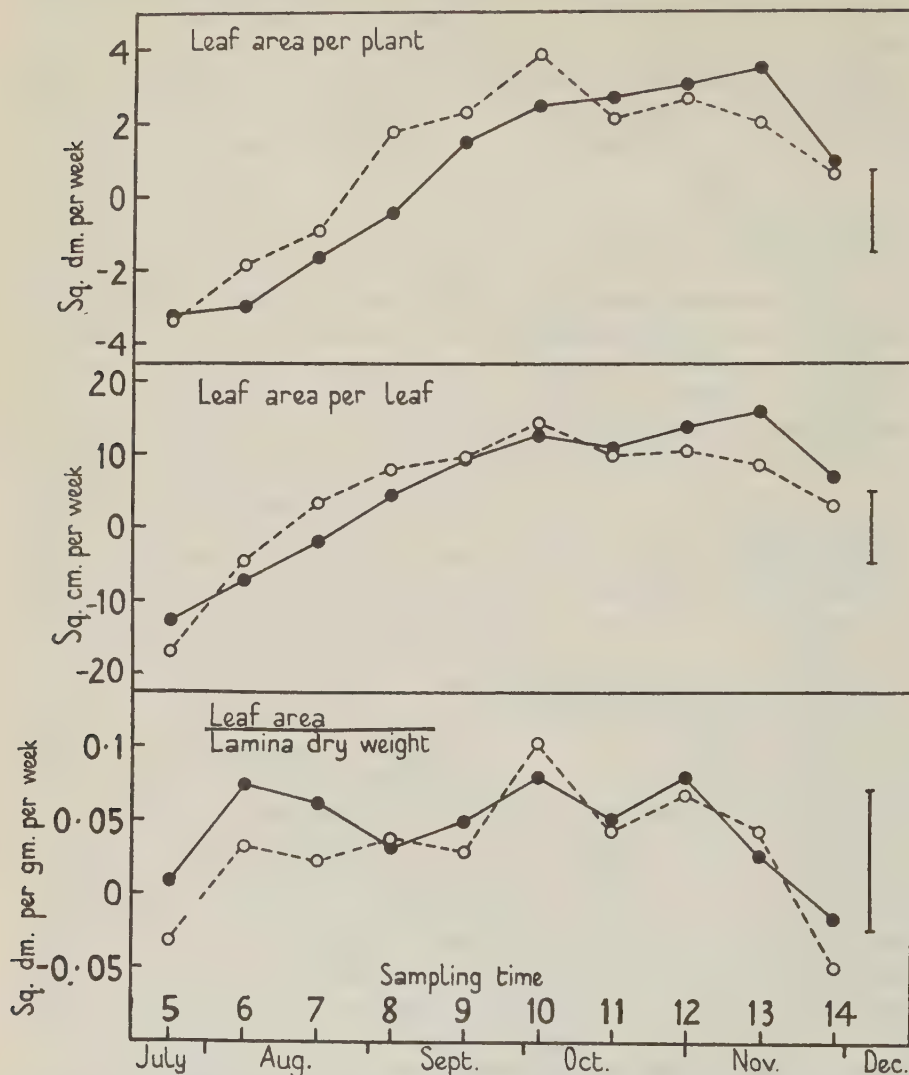


FIG. 12. Linear regression coefficients on sowing date of leaf area per plant, leaf size and ratio of area to lamina dry weight. Full line, sugar-beet; broken line, mangold. Significant differences shown as vertical lines.

gold. We should expect these variations to be accompanied by variations in the leaf area/leaf weight ratio, and that this is so may be seen from Figs. 11 and 12. If all the leaves belonged to a homogeneous series characterized by a uniform leaf area/lamina dry weight relation, we should expect that an

increase in lamina dry weight would be accompanied by a decrease in leaf area/lamina dry weight ratio. Deviations from this expectation imply a real effect on the leaf area/lamina dry weight ratio other than that associated with variations of lamina dry weight per leaf in a homogeneous population.

The mean ratio for all sowing dates (Fig. 11) was lower in the middle of the experimental period than at the beginning and end. The form of the curve was the inverse of the curve of mean lamina dry weight per leaf, that is to say, the ratio had high values when the lamina dry weight was low and vice versa. The time changes in the ratio were, therefore, in agreement with the hypothesis of homogeneity.

The ratio was greater in mangold than in sugar-beet, at those times when the lamina dry weight per leaf (Fig. 10) was also greater in mangold. Though for both, the ratio decreased with increase in lamina dry weight, it was greater for a given lamina dry weight in mangold than in sugar-beet, and for this reason the difference between the two crops in leaf area per plant was smaller than their difference in lamina dry weight. The tendency for the difference between the ratios for mangold and sugar-beet to increase in the later stages of growth is in accordance with the observed decrease with time in the superiority of mangold to sugar-beet in mean lamina dry weight per leaf.

The linear regression coefficient of the leaf area/lamina dry weight ratio on sowing date (Fig. 12) was positive over the whole growth period except at the last sampling time, and for mangold at sampling time 5. In the early stages of growth, later sowing depressed the mean lamina dry weight per leaf, so that an increase in the leaf area/lamina dry weight ratio with later sowing would be expected at this time. But later the lamina dry weight per leaf increased with later sowing, and as the effect on the leaf area/lamina dry weight ratio continued to be positive, it must be concluded that the relation between the leaf area/lamina dry weight ratio and lamina dry weight per leaf was modified by varying the sowing date in the sense that leaves of a given lamina dry weight had a greater leaf area/lamina dry weight ratio. This might be taken to imply that the thickness of leaves of a given area or given weight was less in late sowings than in early, but this is not necessarily so, for the reduction in dry weight per unit area with late sowing might be accompanied by an increase in water content. Although the water content of the lamina was increased by late sowing, precisely the same effects were found in the variations of leaf area/lamina fresh weight ratio and lamina fresh weight per leaf, so that the conclusions are not upset by the consideration of water content. The simplest statement of this result appears to be, therefore, that later sowing caused the production of larger but thinner leaves than early sowing.

It follows that as the leaf area/lamina dry weight ratio showed considerable variation and was dependent not only on mean lamina dry weight but also on crop and sowing date; the lamina dry weight per plant was not a simple measure of leaf area, and this also applies to lamina fresh weight. Ballard and Petrie (1936) have calculated Unit Leaf Rate on a leaf dry weight basis, and

it is clear from the present results that Unit Leaf Rate calculated in this way must bear a complex relation to Unit Leaf Rate computed on the usual basis of leaf area.

6. Analysis of growth.

In comparing the rate of growth of a crop at different stages, it is ideally desirable to use a measure of growth which is independent of the size of the plant, that is to say, to measure growth as a rate of increase per unit of growing material. A first approximation to such a measure is the *Relative Growth Rate* or *Efficiency Index*, defined as the relative rate of increase of dry weight, which was suggested by Blackman (1919).

The Relative Growth Rate may be resolved into its two components (1) the rate of increase of dry weight per unit leaf area, or *Unit Leaf Rate*, and (2) the leaf area per unit dry weight of plant, or *Leaf Weight Ratio*. The Leaf Weight Ratio is important since it represents the proportion which the assimilating surface bears to the size of the whole plant. This method of growth analysis was developed by Gregory (1917) and West, Briggs, and Kidd (1920).

In this section the variation of the three growth functions, Relative Growth Rate, Unit Leaf Rate, and Leaf Weight Ratio, will be examined.

Fisher (1921) has shown that the Relative Growth Rate may be accurately estimated by the following method independent of the form of the growth curve. In the interval between times t_1 and t_2 the mean dry weight per plant is given by $\frac{W_2 - W_1}{\log_e W_2 - \log_e W_1}$, where W_1 is the dry weight per plant at time t_1 and W_2 that at time t_2 . The increase in dry weight per unit time in the same interval is $\frac{W_2 - W_1}{t_2 - t_1}$, so that the Relative Growth Rate is given by:—

$$\frac{W_2 - W_1}{t_2 - t_1} \div \frac{W_2 - W_1}{\log_e W_2 - \log_e W_1} = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1}.$$

Similarly the mean leaf area per plant is given by $\frac{L_2 - L_1}{\log_e L_2 - \log_e L_1}$, where L_1 is the mean leaf area per plant at time t_1 , and L_2 that at time t_2 . Hence, the

Unit Leaf Rate is $\frac{W_2 - W_1}{t_2 - t_1} \times \frac{\log_e L_2 - \log_e L_1}{L_2 - L_1}$ and the mean Leaf Weight

Ratio is $\frac{L_2 - L_1}{\log_e L_2 - \log_e L_1} \times \frac{\log_e W_2 - \log_e W_1}{W_2 - W_1}$. Thus the Relative Growth Rate

is the product of Unit Leaf Rate and Leaf Weight Ratio.

(a) *Relative growth rate.* The calculated values of the Relative Growth Rate, expressed in units of gm. per gm. per week, for the mean of all sowings of sugar-beet and mangold are plotted in Fig. 13. The R.G.R. fell steadily throughout the whole sampling period for every sowing, i.e. from approximately the sixth leaf stage, at which the plants were thinned, and the rate of

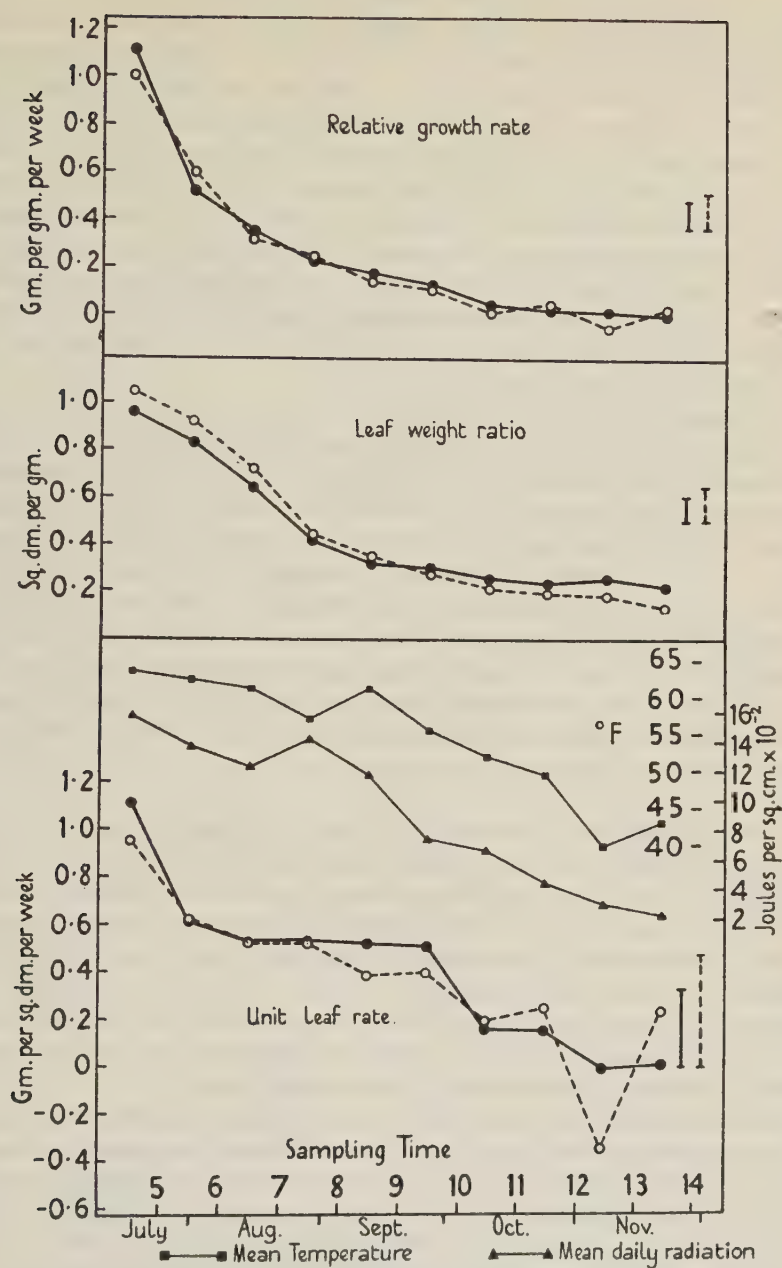


FIG. 13. Relative growth rate, leaf weight ratio, and unit leaf rate. Means of all sowings. Full line, sugar-beet; broken line, mangold. Significant differences shown as vertical lines.

fall decreased with time so that the curves are concave upwards, falling to zero at the end of the period. The prolonged fall of R.G.R. in the later stages of growth is similar to that found for other plants, e.g. barley (Gregory, 1926), maize (Briggs, Kidd, and West, 1920), wheat, oats, and Sudan grass (Ballard and Petrie, 1936) (Heath, 1937*c*). Briggs, Kidd, and West (1920) have shown in their analysis of Kreusler's data for maize that during germination, before assimilation begins, the R.G.R. has negative values, subsequently rising rapidly. The initial phase of increasing or steady R.G.R. was completed in the present experiment before the sampling began. Over the whole period, the mean value for sugar-beet was slightly but significantly greater than the mean for mangold, and though some intersection of the curves is evident, no significant variation of the difference between sugar-beet and mangold was found.

The variation of R.G.R. with sowing date is shown by the graphs of the linear regression coefficient in Fig. 14. These have the same form as the curves of the means for all sowing dates, being large and positive at first and falling later to zero, so that the magnitude of the fall over the whole period was greater the later the date of sowing. A similar result would be expected if the downward drift of R.G.R. was identical for all sowings, and determined by internal factors of age, but if this were so, the regression coefficients on sowing date, when sowings are compared on a time scale relative to sowing date, should be zero. Actually, negative values becoming smaller towards the end of the growth period were found, showing that the R.G.R. was dependent both on internal factors of age and on external factors. There was no significant difference between the regression coefficients for sugar-beet and mangold.

(b) *Leaf weight ratio.* The values of the Leaf Weight Ratio in sq. dm. per gm. of plant for the means of all sowings are given in Fig. 13. The graphs are similar to those of R.G.R., falling throughout the whole period, but not to zero. The fall was steeper in mangold than in sugar-beet, so that the curves intersect in the middle of the period, and although this did not appear in the Analysis of Variance as a significant variation with time of the difference between sugar-beet and mangold, there is no doubt of the reality of the effect; it is the expression of the more rapid decline of leaf area in mangold than in sugar-beet which has already been pointed out.

The regression of L.W.R. on sowing date (Fig. 14) was positive and declining throughout the period, and there was no difference between the values for sugar-beet and mangold. The increase in L.W.R. with later sowing is attributable partly to a decline of L.W.R. with age and partly to the effect of later sowing in increasing the leaf area. When age differences were eliminated by comparing the sowings on a time scale relative to sowing date, the regression coefficients showed a continued rise from negative to positive values. There does not appear to be any obvious explanation of the initial negative values, but the upward trend is the consequence of the more prolonged increase, and

ultimately greater magnitude, of leaf area in the later sowings than in the early. The differential effect on L.W.R. of sowing date is not very great since the large leaf area of the later sowings occurs late in the life history when the plant is large.

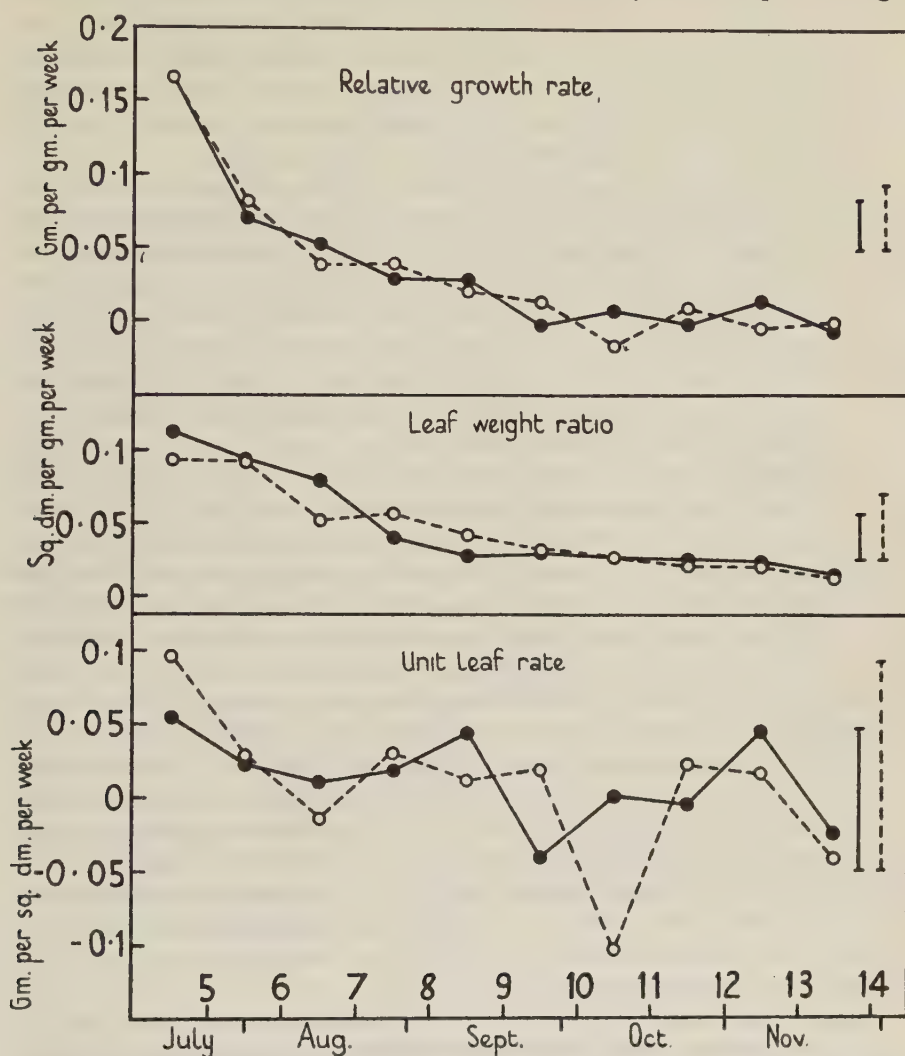


FIG. 14. Linear regression coefficients on sowing date of relative growth rate, leaf weight ratio, and unit leaf rate. Full line, sugar-beet; broken line, mangold. Significant differences shown as vertical lines.

(c) *Unit leaf rate.* The Unit Leaf Rate expressed in gm. per sq. dm. per week for the mean of all sowings is plotted in Fig. 13. It showed a fall throughout the period similar to that found for R.G.R. and L.W.R., but more nearly linear. The mean value for sugar-beet was slightly greater than that for mangold, and the difference between the two did not vary significantly with

time. No significant relation of U.L.R. to sowing date was found. When the sowings were compared on a scale of calendar time (Fig. 14), the linear regression coefficients of U.L.R. on sowing date were small and with one exception positive, and on a time scale relative to sowing date they were consistently negative. The effect of age on the U.L.R., caused by variation of the sowing date, was clearly small, and consequently it is difficult to attribute the fall of U.L.R. over the experimental period to internal factors decreasing photosynthetic efficiency of leaves with advancing age.

Gregory (1926), working on barley grown in pot culture, concluded that 'net assimilation rate (i.e. U.L.R.) is almost completely controlled by the factors of temperature and radiation, and internal factors play a minor part' in the early stages. Briggs, Kidd, and West (1920) found for maize that the U.L.R. was more closely correlated with temperature than with any of the other environmental factors. In the later stages, however, their U.L.R. curves differed from those found by Gregory, Ballard and Petrie (1936) and those in the present experiment, in that there was a prolonged phase during which U.L.R. showed no obvious downward trend.

An attempt was made to establish a relation between U.L.R. and mean daily radiation and mean temperature, using the mean values of U.L.R. for all sowing dates. The three variates are plotted together in Fig. 13. Highly significant regressions of U.L.R. on radiation and on temperature were found, but a linear regression on time alone gave an equally good fit. A multiple regression on the three factors, time, radiation, and temperature together, accounted for very little more of the variance of U.L.R. than a regression on time alone, so that the radiation and temperature terms were not significant. All three factors were so highly correlated that it was impossible to distinguish between them. This may be the consequence of a smoothing out of the U.L.R., radiation, and temperature curves, owing to the rather long interval of a fortnight between observations. No definite correlation of U.L.R., radiation, and temperature, apart from the linear fall of all three, could be demonstrated. In view of the absence of any significant variation of U.L.R. with age differences due to variation of sowing date, however, it appears probable that the fall of U.L.R. with time was caused by the fall of radiation or temperature, and not by a change in internal factors of age.

The negative value of U.L.R. for the interval between samplings 13 and 14 (Fig. 13) was due to loss of leaves by frost damage, but this was a secondary temperature effect, different in nature from a normal temperature variation of U.L.R. attributable to alteration of the rate of photosynthesis or respiration.

(d) *Relative leaf growth rate.* Gregory (1926) has made use of another measure of growth, the *Relative Leaf Growth Rate*, defined as the rate of in-

crease of leaf area per unit leaf area, and calculated as $\frac{\log_e L_2 - \log_e L_1}{t_2 - t_1}$ in analogy with the Relative Growth Rate. This function may be considered as

the product of Unit Leaf Rate and the ratio $\frac{L_2 - L_1}{W_2 - W_1}$ which is a measure partly of the distribution of added dry matter between the leaves and the rest of the plant, and partly of the efficiency with which the increased dry matter of the leaves is utilized in increasing the leaf area. Alternatively it may be considered as the resultant of changes in leaf number, and in the mean area per leaf. From this point of view the Relative Leaf Growth Rate is determined by the activity of the meristem in producing new leaves, the loss of leaves by senescence and death, and the rate of expansion of the young leaves produced by the meristem. These three processes are likely to be determined by different internal factors and may be affected quite independently by climatic factors. Increase in total dry weight is determined by the product of leaf area and the assimilation rate of the leaf surface. The latter factor is measured by Unit Leaf Rate, and variation in the former is measured by the Relative Leaf Growth Rate.

The Relative Leaf Growth Rate was calculated from the data of the present experiment, but the results give no information additional to that which has already been described. It was found that the Relative Leaf Growth Rate fell rapidly at first, and from the interval between sampling times 6 and 7 onwards it differed little from zero. There was no significant difference between sugar-beet and mangold, but during the later stages the values tended to be higher in sugar-beet. Later sowing caused a marked increase at first, for reasons similar to those put forward with regard to Relative Growth Rate (p. 472). Gregory found for barley, that after the elimination of a complex time trend by a graphical method, the Relative Leaf Growth Rate showed a positive correlation with day temperature and negative correlations with night temperature and total radiation. For all sowings a linear regression of the mean Relative Leaf Growth Rate on time, mean temperature, and mean daily radiation was fitted to the data of the present experiment, but only the time term was significant. The absence of any apparent correlation with radiation or temperature may be due to the comparatively small deviations of these factors from smooth trends.

DISCUSSION

The sequence of changes in dry matter was very similar in sugar-beet and mangold; the major difference lay in the distribution of dry matter between lamina, petiole, and root. Table VIII shows that a greater proportion of assimilate was translocated to the root in mangold than in sugar-beet. The later sampling intervals are omitted from the table, because the dry weight increases were small compared with their errors so that the percentage increases were very inaccurately determined.

As a result, the sugar-beet plants developed a greater leaf area than the mangold, but the difference between the two in this respect was reduced by the smaller utilization of elaborated material in the petiole and by the

production of a greater leaf area per gm. of dry matter in the leaf lamina in mangold. The photosynthetic efficiency of the leaves as measured by the Unit Leaf Rate was only slightly greater in sugar-beet, and the ultimately greater production of dry matter in sugar-beet was mainly due to its superiority in leaf area. This superiority was gained by the production of more leaves, for over the greater part of the life cycle the leaves of the mangold were larger than those of sugar-

TABLE VIII

Increase in Dry Weight of Lamina, Petiole, and Root as Percentage of Total Dry Weight Increase. Mean of all Sowings.

Sampling interval.	Sugar-beet.			Mangold.		
	Lamina.	Petiole.	Root.	Lamina.	Petiole.	Root.
5-6	22	22	56	24	14	62
6-7	20	20	60	14	12	74
7-8	15	20	65	14	10	76
8-9	14	24	62	-4	2	102
9-10	7	20	73	0	2	98

beet. These facts suggest that the differences in growth between the two plants were the result of a greater facility for the movement of assimilate from leaf to root in mangold than in sugar-beet. The slightly greater production rate and markedly smaller death rate of leaves of the main axis, together with the development of more axillary leaves, seem to indicate that in the partition of assimilate between stem and root meristems, the sugar-beet stem has an advantage compared with that of the mangold. It seems probable that the source of this advantage lies in a greater ability of the leaves and meristems of the shoot to utilize the products of photosynthesis in sugar-beet than in mangold, but on the other hand it is possible that the cause is to be found in a less efficient translocatory system, or a smaller ability of the root of sugar-beet to utilize assimilate.

It is clear that the greater accumulation of sucrose in the root of the sugar-beet is not a simple result of the production of an excess of carbohydrate material in photosynthesis, for though the sugar-beet produced slightly more total dry matter, the dry weight of root was actually less than in mangold. The source of any differences in sugar content between the two plants must lie in properties which determine the extent to which the products of assimilation are elaborated to form more complex compounds, and not in a difference of the total amount of available assimilate.

The effect of time of sowing on growth was also the result of a variation in the distribution of assimilate between the leaves and the root. This is shown in Table IX.

As the plants aged, the proportion of the total dry weight increase appearing in the root became greater, but the difference between sowings cannot be explained as merely a difference of age, for ultimately the later sowings developed a greater dry weight of lamina and petiole than the early sowings

although the total dry weight was less. Independently of age, later sowing induced a greater growth of lamina and petiole. It has been shown that this was effected by an increase in mean leaf size accompanied by a slightly greater rate of increase of leaf number. The larger leaves of the later sowings were also thinner, so that the effect of sowing date on leaf area was even greater than on leaf weight.

TABLE IX

Increase in Dry Weight of Lamina, Petiole, and Root as Percentage of Total Dry Weight Increase. (Mean of Sugar-beet and Mangold.)

Sampling interval.	Mean of first three sowings.			Mean of last three sowings.		
	Lamina.	Petiole.	Root.	Lamina.	Petiole.	Root.
5-6	13	17	70	45	20	35
6-7	11	13	76	28	24	48
7-8	5	8	87	23	21	56
8-9	-7	0	107	10	19	71
9-10	4	14	82	5	10	85

It is not possible to determine from the present data the factors which caused the increased leaf growth with later sowing. It seems unlikely that the effect could have been induced in the seeds before germination, by keeping them for a slightly longer time in the dormant condition. Nor can it be attributed to climatic factors acting during the time of expansion of the leaves produced towards the end of the growth period, which showed the effect most markedly. In these later stages of growth, leaves initiated at the same time in early and late sown plants, developed to a much greater size in the late sown plants although the climatic factors during their growth were identical for all sowings. It appears, therefore, that the effect must be attributed to internal factors which were induced by environmental factors at, or soon after, germination, such as, for instance, a rapid production of the root system at that time. Conditions during early growth must have had an enduring influence on the growth rates of leaf and root, such that in the late sown plants relatively greater growth occurred at the stem apex throughout their subsequent growth than in those sown earlier. What determines these meristematic activities can at present only be a matter of speculation. Table X shows that the later sowings germinated at higher temperatures, in longer days and under higher light intensities than the early sowings. The soil conditions of water supply and nutrient supply must also have varied with sowing date.

The fact that this effect on leaf growth has been observed in subsequent years (Rothamsted Exp. Station Rep., 1935, 1936) suggests that it is related to the general seasonal trend of climatic factors, rather than to any special deviations from this trend in the spring of 1934.

Whatever the cause of the effect of sowing date on leaf growth, its practical result on yield as measured by dry weight is to introduce a compensation for

late sowing, for since the photosynthetic efficiency per unit leaf area (the Unit Leaf Rate) was as great for the late sowings as the early, the greater leaf area developed by the later sowings tended to reduce the difference between sowings in dry weight caused by variation in the length of the growth period.

TABLE X

Sowing date	Means for four weeks after sowing date.					
	I.	II.	III.	IV.	V.	VI.
Mean hours of daylight per day	14.2	15.1	15.8	16.3	16.6	16.5
Mean temperature (°F.)	48.3	49.7	52.7	56.3	58.9	57.0
Mean radiation per hour of daylight. Joules per sq.cm.	77.4	95.9	113.5	107.6	94.5	104.6

Field experiments carried out at Rothamsted and Woburn in 1935 and 1936 show similar results in the effect of sowing date on the relative growth of tops and roots (Rothamsted Ann. Rep. 1935, 1936).

SUMMARY

A study was made of the growth of sugar-beet and mangold sown on six occasions at intervals of a fortnight in 1934. Samples were taken at fortnightly intervals after thinning to determine (1) dry weight of lamina, petiole, and root; (2) water content of lamina, petiole, and root; (3) leaf area per plant. On a subsidiary series of samples the leaves were marked as they developed, to enable the rates of production and death of leaves to be measured.

Samples were also taken for sugar estimations, the results of which will be discussed in a later paper.

Sugar-beet ultimately attained a greater dry weight than mangold, the difference being mainly in the leaves, for the dry weight of root in sugar-beet was slightly smaller than in mangold. The higher sugar content of the root of sugar-beet than of mangold cannot, therefore, be attributed to the translocation of more carbohydrate; it must be due to a difference between the plants in the extent to which carbohydrate is utilized in growth and in the elaboration of more complex compounds. The dry weight of lamina and petiole continued to increase for a longer period in sugar-beet than in mangold.

Later sowing decreased the dry weight of root at all sampling times, but in the later stages of growth it caused a marked increase in lamina and petiole dry weights.

The water content of mangold was very much greater in all parts of the plant than in sugar-beet. Later sowing caused an increase of water content. The leaf lamina showed a marked fall of water content during the day; there was evidence of a similar but smaller variation in the petiole, but none in the root. Variation of water content between sampling times in all parts of the plant was well fitted by a double regression on accumulated rainfall and time, indicating that it was caused by variation of soil moisture content.

The number of leaves per plant was greater in sugar-beet than in mangold

owing to a slightly higher rate of production and a lower death rate. Later sowing increased the rate of production, so that the difference in leaf number between sowings diminished with time, particularly in mangold, but the number of leaves per plant did not increase with later sowing at any time. The effect of sowing date in increasing leaf dry weight was therefore due to an increase in the size of individual leaves and not of leaf number. The rate of leaf production was correlated with mean temperature, Q_{10} being 3.1. Low temperatures appeared to have an after-effect, for the rate of leaf production in a subsequent period was greater than would be expected from the rise of temperature. No correlation of leaf death rate with any meteorological factor was detected.

The variation of leaf area per plant was similar to that of lamina dry weight. The effect of sowing date on leaf area was greater than on leaf weight, for it was found that the larger leaves of the later sowings had a greater area per unit dry weight, i.e. were thinner. It is probable that the effect of varying sowing date on leaf growth is determined by environmental factors at, or soon after, germination.

The Relative Growth Rate was slightly greater in sugar-beet than in mangold; it decreased throughout the growth period, falling eventually to zero. This was due to a decline both in the Leaf Weight Ratio and the Unit Leaf Rate. The Leaf Weight Ratio fell off less rapidly, and the Unit Leaf Rate was greater, in sugar-beet than in mangold.

Later sowing increased the Relative Growth Rate in the early stages, and this was due to an increase in the Leaf Weight Ratio, for the Unit Leaf Rate was unaffected by date of sowing.

Both mean temperature and mean daily radiation showed a fall over the experimental period, similar to that of the Unit Leaf Rate, but no significant correlation of either of these factors with Unit Leaf Rate, after elimination of time trend, could be demonstrated.

The primary data have been deposited in the Natural History Museum, South Kensington.

The authors wish to record their indebtedness to Mr. S. A. W. French for much assistance with the statistical computation.

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Mycorrhiza in *Tropaeolum majus* L. and *Phlox Drummondii* Hook

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With seven figures in the Text.

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INTRODUCTION

THE mycotrophic habit is proved to be of wide occurrence in many families of plants. Modern researches show that the presence of the mycorrhizal fungus is not essential for the seedling development of the higher symbiont (Melin, 1922; Knudson, 1927-33; McLennan, 1935). Knudson (1929) related the abnormal root development of the aseptid seedlings of *Calluna vulgaris*, as stated by Rayner (1915), as due to either the toxicity of the nutrient solution or the mercuric chloride used for seed sterilization.

Very few attempts have been made to isolate the mycorrhizal fungus or to synthesize mycorrhiza. Rhizoctonia species were isolated from various orchid plants; synthetic experiments were made successfully in most cases. McLuckie and Burges (1932) isolated Rhizoctonia species from *Eriostemon Crowei*. Rhizoctonia species were also isolated from a number of different flowering plants; the form, isolated from wheat, has been successfully reinoculated into aseptid seedlings. Phoma species were isolated from Ericaceous and Epacridaceous plants as well as from *Pellia epiphylla* and *Fragaria chiloensis*. Fusarium species were isolated by Asai (1934) from different Japanese plants. Sterile fungi had been isolated by Freisleben (1934) from different *Vaccinium* species.

MATERIAL AND METHOD

The roots of many plants growing in the Zaafran Palace garden, Cairo, were examined for the presence or absence of mycorrhiza. The roots were

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cut and stained with lactophenol cotton blue. The presence of fungal hyphae was detected in the following species: *Tropaeolum majus L.*, *Phlox Drummondii* Hook., *Verbena officinalis L.*, and *Clerodendron inerme* Geartn. Only *Tropaeolum majus* and *Phlox Drummondii* come under detailed study in this paper. To carry on the investigations during the summer season, seeds were grown in pots containing soil from the winter beds.

For microtome sections, the fixatives 2B and 2BE (La Cour, 1931) were used. Some sections were bleached with hydrogen peroxide, others were left untreated to study the distribution of fats. Thionin and orange G (Stoughton, 1930), as well as lactophenol cotton blue (Hough, 1930), were used as stains.

MYCORRHIZA IN *TROPAEOLUM MAJUS*

Since soil conditions were proved to be of great importance in determining the parasitic and symbiotic tendencies of the endophytic fungus (Rayner, 1921 and 1934; Reed and Fremont, 1934 and 1935), a study of the soil conditions seems to be essential. The soil samples were taken at different levels; the pH value, the carbonate content, as well as the humus content, were determined as shown in the following table:

Sample.	pH value.	Carbonate (%).	Humus (%).
1	7.8	3.20	3.10
2	7.6	3.08	3.32
3	7.6	3.08	3.11
4	7.8	3.20	3.14
Mean	7.7	3.14	3.17

The pH value is high, the soil being alkaline; the humus content is definitely low.

Tropaeolum majus shows a well-developed endotrophic mycorrhiza (Fig. 1). The fungus gains entrance through the piliferous layer (Fig. 2). The outer mycelium is usually septate with dense protoplasmic contents; it increases in thickness and the transverse septa disappear as the fungus grows deeply in the cortex. However, the fungus retains occasionally its transverse septa. The hyphae found at the outer cortical layers are usually rich in contents; while those situated in the deep layers are highly vacuolated and have poor contents. The tips of certain hyphae swell and form vesicles which are not delimited by transverse septa from the parent hyphae (Fig. 3). The vesicles are usually distributed all over the cortex; they are commonly seen in the intercellular spaces and occasionally inside the cells. Young vesicles have no vacuoles; they become at later stages of development highly vacuolated with shrinkage of their contents.

The fungus forms mainly in the innermost cortical layer, very rarely in the cortical layer next to it, complete networks of very fine hyphae. These structures or arbuscules appear as brownish masses in the living material; they occur as side branches of the intracellular hyphae (Fig. 4) and are not terminal. At a later stage, the hyphae forming the network body lose their

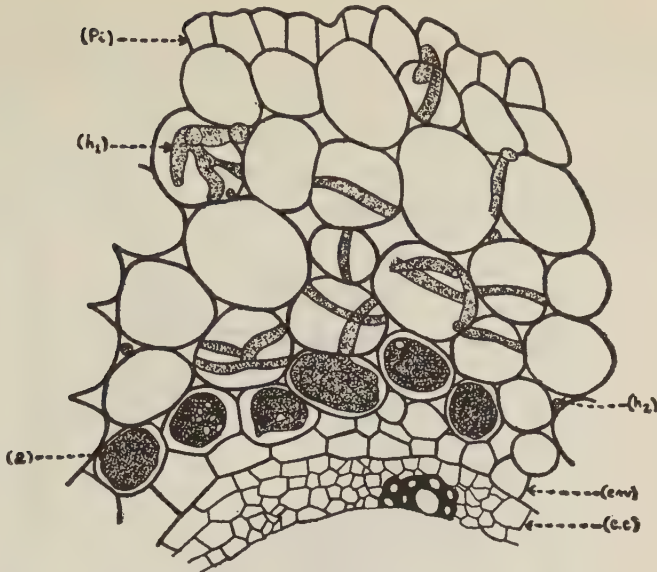


FIG. 1. Part of transverse section of root of *Tropaeolum majus* showing the piliferous layer (*Pi*), intracellular and intercellular hyphae (*h*₁ and *h*₂), arbuscules and sporangioles (*a*), endodermis (*en*), and central cylinder (*c.c*). $\times 255$.

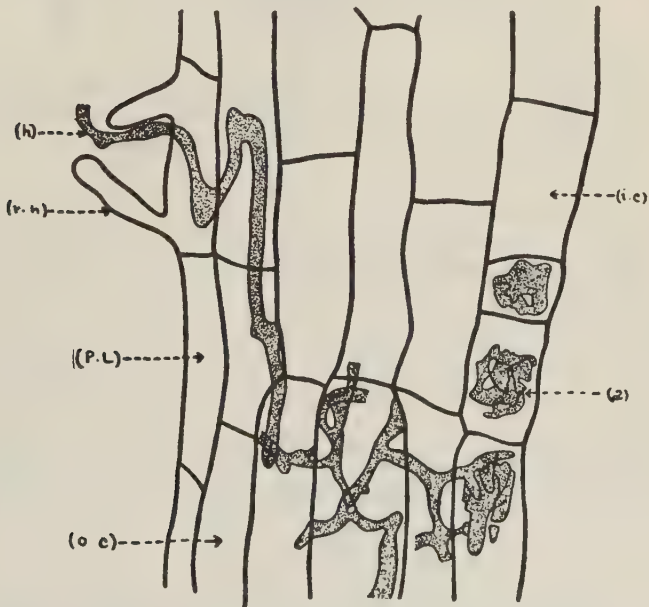


FIG. 2. Radial longitudinal section of the cortex of *Tropaeolum* root showing root-hairs (*r.h*) and ingress of the fungus (*h*) by the piliferous layer (*P.L*), arbuscules (*a*) in different stages of digestion, outer (*o.c*) and inner (*i.c*) cortical cells. $\times 235$.

sharply defined structures and form sporangioles (Fig. 5). The host nuclei within the sporangioles undergo much hypertrophy with occasional change in shape.

MYCORRHIZA IN *PHLOX DRUMMONDII*

The mycotrophic habit in *Phlox Drummondii* does not differ very much from that of *Tropaeolum majus*. However, the fungus forms most commonly

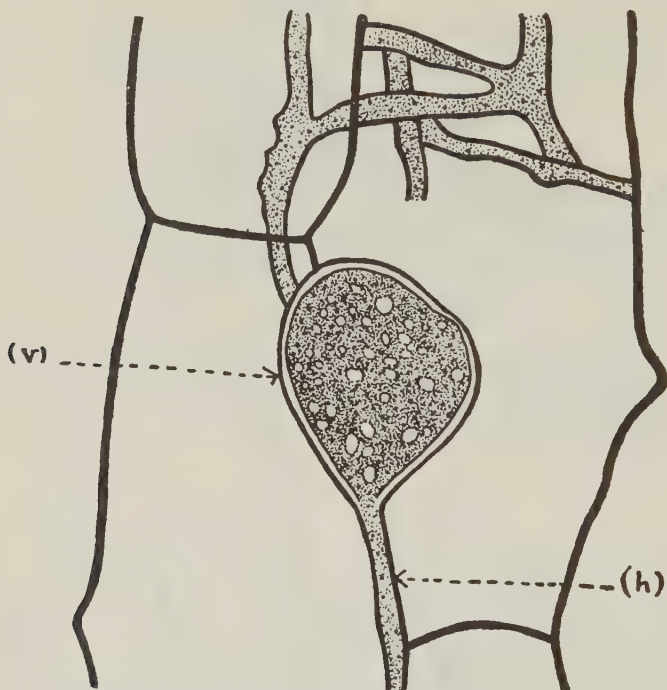


FIG. 3. Longitudinal section of the cortical cells of *Tropaeolum* root showing an inter-cellular hypha (*h*) bearing a vesicle (*v*) much vacuolated. $\times 687$.

intracellular hyphal clumps in addition to the usual arbuscules. The vesicles are very rarely met with and show occasionally transverse septa. The mycelium is always intracellular and the compound arbuscules are localized in definite layers.

In both *Tropaeolum majus* and *Phlox Drummondii* no trace of the fungus has been seen in the aerial portions of the plant.

ISOLATION EXPERIMENTS

The young roots, after being washed thoroughly in running tap-water, were transferred into 1 : 1,000 mercuric chloride solution for a few seconds up to two minutes. They were then repeatedly washed in sterilized water and dried on sterilized cotton-wool. The sterilized roots were cut into small fragments

and transferred into Petri dishes containing different media. The media used (Fred and Waksman, 1926) were Richard's solution agar, Brown's starch synthetic agar, malt extract agar, raisin extract agar, and carrot extract agar. Malt extract agar was acidified by adding a few drops of N/10 HCl so as to be

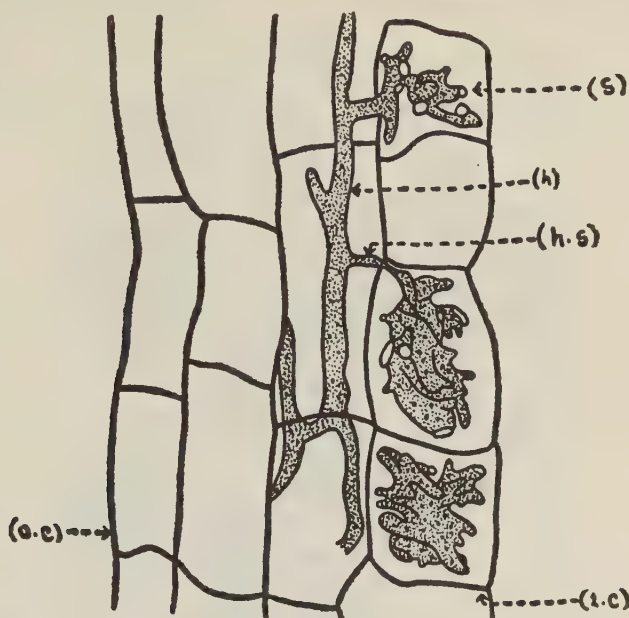


FIG. 4. Radial longitudinal section of the cortical cells of root of *Tropaeolum* showing outer (*o.c*) and inner (*i.c*) cortical cells, arbuscules connected to the intracellular hyphae (*h*) by a side branch (*h.s*), and an arbuscule (*s*) in early stage of formation. $\times 407$.

on the acidic side to eliminate bacterial growth. Media were autoclaved for twenty minutes at a pressure of 1.5 atmospheres.

The Petri dishes, containing the plant material, were incubated at 25° C. Hyphal tips were isolated by a sterilized needle and were transferred into slants of raisin, malt, and carrot extract agar.

Fungi isolated from Tropaeolum majus.

In the material which was treated in the corrosive sublimate for a few seconds, hyphal growth could be recognized after twenty-four hours incubation. Among the fungi isolated were *Aspergillus flavus*, *Aspergillus terreus*, *Penicillium expansum*, *Penicillium wortmani*, *Penicillium meleagrinum*, *Chaetomium botrychoides*, *Fusarium solani*, *Acrothecium lunatum*, and *Alternaria fasciculata*.

From the material treated with the sublimate from thirty seconds up to two minutes sterile mycelium was obtained; while in the material left for more than two minutes, no fungal growth was to be recognized within five to six

days. The sterile mycelium began to appear after the second day of incubation and sometimes after the third or fourth day. The reason that the sterile mycelium was not isolated in the first case may be due to the fact that this fungus grows slowly and it is overgrown by the other fungi.

The sterile mycelium was grown on several media in Petri dishes and in

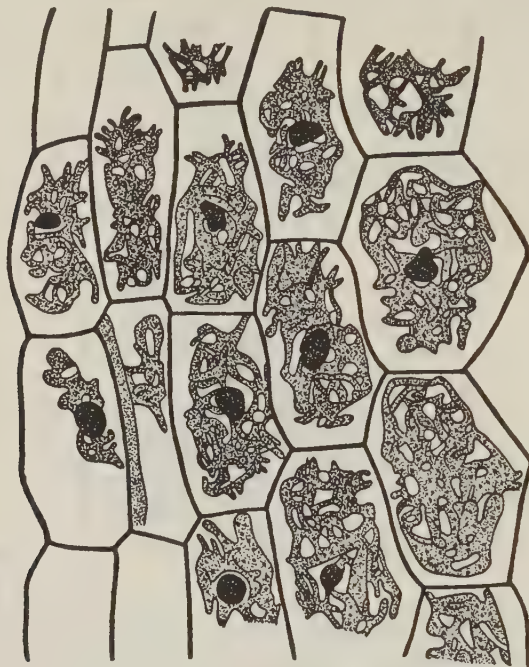


FIG. 5. Tangential longitudinal section of root cortex of *Tropaeolum* showing arbuscules and sporiangioles in various stages of digestion. $\times 466$.

test-tube slants and plugs. It was examined both macroscopically and microscopically. On malt extract agar in Petri dishes the colony is white with characteristic bracket-like patches and growth is fairly rapid. On oat-meal tube, after seven days, the colony becomes white, woolly, and elevated in the centre. On raisin slant it grows very slowly and the colony is usually brownish in colour. On potato extract agar the mycelium grows slowly and the colony is whitish in colour with elevated centre; the characteristic bracket-like patches developed on malt extract agar are also recognized here. On carrot extract agar the mycelium shows vigorous growth; young colonies are woolly and whitish in colour; at a later stage the colony becomes greyish in colour and the medium itself becomes also grey.

The microscopic examination of the fungus on the above media shows no spores or sclerotia. On raisin extract agar the mycelium is brown in colour and shows many swollen cells (Fig. 6). The cells are multinuclear, much

vacuolated, and rich in oil globules. The swollen cells show less vacuolation and richer fatty contents. The ordinary cells are about $30.8\ \mu$ to $11.5\ \mu$ long and about $3.9\ \mu$ wide. The swollen cells are about 9.8 to $5.9\ \mu$ long and 9.8 to $6.9\ \mu$ wide.

Fungi isolated from Phlox Drummondii.

In the roots treated with the corrosive sublimate for a few seconds hyphal growth was seen on the second and third days of incubation. The fungi



FIG. 6. Part of the mycelium of the fungus isolated from root of *Tropaeolum majus*. $\times 510$.

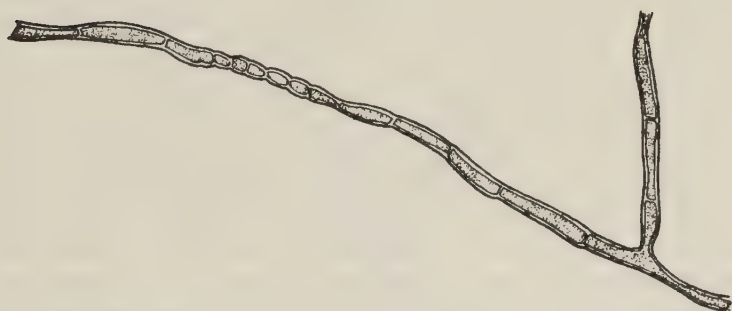


FIG. 7. Part of the mycelium of the fungus isolated from root of *Phlox Drummondii*. $\times 653$.

isolated were: *Penicillium africanum*, *Penicillium meleagrinum*, *Fusarium scirpi*, and *Alternaria fasciculata*.

In the roots which were treated in the mercuric chloride solution for a time ranging from a few seconds up to thirty seconds, a fungus belonging to *mycelia sterilia* was isolated in most cases. It shows vigorous growth and orange-red colour on carrot extract agar; it causes the medium to change into red colour. It gives no spores on different media including raisin extract agar, potato extract agar, malt extract agar, and oatmeal agar. A part of the mycelium from carrot extract agar is shown (Fig. 7).

CULTIVATION EXPERIMENTS

The cultures used were either Rayner's nutrient agar (Rayner, 1915) or sand culture moistened with Melin's nutrient solution (Melin, 1922). The flasks were either plugged with cotton-wool in the ordinary way or a Berkefeld filter (Åslander, 1930) was used. The cultures were autoclaved for twenty minutes at 1.5 atmospheres on three consecutive days.

The seeds were transferred into 0.25 per cent. mercuric chloride solution and subjected to exhaustion for five minutes to remove air bubbles from around the seeds and to allow the disinfectant to penetrate through. The seeds were left in the disinfectant for five minutes more after exhaustion. The seeds, after being passed through a series of sterilized water for washing, were transferred into Petri dishes containing 1.2 per cent. agar in sterilized water and left for about three to four days. Contaminated seeds were rejected, while seeds remaining free from contamination were transferred into the sand or agar culture.

The *Tropaeolum majus* seedling, raised from sterilized seeds, grow normally and show good development of their root system in both the sand and agar cultures. The aseptic seedlings were removed from the cultures and longitudinal sections were made in their roots; no trace of fungal hyphae was found. The aseptic seedlings show in advanced stages of germination slight withering and yellowing of their leaves. This fact suggests the possibility that these symptoms may be due either to the absence of the fungus or to the partial toxicity of the mercuric chloride. To test this idea, the following experiment was made. Sterilized and unsterilized seeds were sown separately on sand cultures in the same time and under the same cultural conditions. The seedlings, raised from sterilized seeds, showed the usual withering and yellowing of their leaves in advanced stages of germination; while seedlings of similar age raised from unsterilized seeds showed vigorous growth and large green leaves. Both seedlings were removed and sections were made in their roots; no trace of the fungus was found in either cases. Thus, it seems that the mercuric chloride is slightly toxic to the seed.

Experiments on synthesis of host and parasite were made with both *Tropaeolum majus* and *Phlox Drummondii*. Sterilized seeds of *Tropaeolum majus* were inoculated with the sterile fungus in both sand and agar cultures. The inoculated seeds began to germinate earlier than control seeds. When the roots of the inoculated seedlings were cut and stained, the fungus was found penetrating the root-cells and showed arbuscular formations. When the sterilized seeds of *Phlox Drummondii* were inoculated with the different isolated fungi and the roots of the resulting seedlings examined no trace of a fungus was observed.

DISCUSSION

The soil is definitely alkaline in contrast to the acid soil frequently recorded for mycotrophic plants (Asai, 1934; McLuckie and Burges, 1932). The fungus, outside the piliferous layer of the root, is narrow and septate; it becomes non-septate with an increase in diameter after penetrating the root.

This observation together with those of McLennan (1926) and McLuckie and Burges (1932) suggest the possibility that the phycomycetoid character, acquired by the cortical endophyte, is a secondary character due to the digestive activity of the host cells with subsequent widening of the hyphae and loss of their septa. However, the endophyte occasionally retains its septation. The dimorphism of the mycelium seems to be due to physiological differences in the host cells, rather than to the infection by two distinct fungi. Isolation experiments from *Tropaeolum majus* reveal no fungus of phycomycete nature. The fungus which appears to belong to *mycelia sterilia* is very commonly met with during most isolation experiments. *Aspergillus* and *Penicillium* as well as species of *Chaetomium*, *Fusarium*, *Acrothecium*, and *Alternaria* have been very rarely isolated and are considered as members of the soil epiphytic flora of the roots (Sabet, 1935). These soil fungi may help in the breaking down of the organic material of the soil into water-soluble components available for the plant (Burges, 1936). In summer, the unfavourable season for *Tropaeolum majus* in Egypt, the plant develops weakly while the endophyte grows very well and shows scarcely any trace of arbuscular formation; the endophyte seems to be more parasitic. In winter, the relationship is more of a symbiotic nature (Reed and Fremont, 1934-5).

The mycotrophic habit in *Phlox Drummondii* is facultative with little difference between infected and uninfected plants.

SUMMARY

Both *Tropaeolum majus* and *Phlox Drummondii* showed well-developed endotrophic mycorrhiza when growing as garden plants in a soil which was alkaline with a low humus content. The fungus, outside the piliferous layer, is septate; it becomes non-septate with an increase in diameter after penetrating the root. Aseptic seedlings of *Tropaeolum majus* raised either in sand or agar cultures show a well-developed root system but no fungus. Synthesis between parasite and host succeeded with *Tropaeolum majus*. The fungus appears to belong to the class *mycelia sterilia*.

In conclusion, I wish to express my grateful thanks to Professor Y. S. Sabet for his valuable assistance and criticisms, to Professor F. J. Lewis for his unfailing help, and to A. A. Nayal for useful suggestions.

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Physiological Studies in Plant Nutrition

VIII. The Relation of Respiration Rate to the Carbohydrate and Nitrogen Metabolism of the Barley Leaf as determined by Phosphorus and Potassium Supply

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With nine Figures in the Text

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INTRODUCTION

THE present paper describes the results of an investigation of the interaction between phosphorus and potassium supply in certain important physiological processes of barley leaves, namely respiration rate, and nitrogen and carbohydrate metabolism. The primary object was to extend the observations of Gregory and Sen (1937), who had already investigated the nitrogen-potassium interaction, so as to include all three of the major nutrient elements. The investigation continued throughout the vegetative period in order to reveal interactions with growth-stage, and so give a wider view of the correlations involved. Very great differences in phosphorus and potassium

concentrations were utilized, leading to the production of extreme plant types. The whole of the experimental data are presented, and they may be approached from several points of view. Only one approach is attempted fully in the subsequent analysis, namely, that of the dependence of respiration rate on the intensity of the other factors investigated. But all important modifications of those other factors, due to treatment, are indicated, and in some instances the probable causes of such modification are incidentally referred to.

The method of experimental approach employed may clearly be of considerable value in elucidating the vexed question of the necessity of phosphate in the respiratory processes of higher plants, and from this point of view it is perhaps surprising that its value has not hitherto been sufficiently appreciated. Evidence will be presented that the deficiency method is indeed capable of throwing considerable light on the problem.

EXPERIMENTAL PROCEDURE

As in previous experiments (Gregory and Richards, 1929; Richards and Templeman, 1936), barley, var. Plumage Archer, was grown in sand culture in the open. It was sown in the first week of May, 1933, and treated in the usual manner except for slight modification of the manuring scheme. The total amount of salts applied was only 81 per cent. of that in previous years, in order that the nutrition levels should agree with those adopted in the experiment of Gregory and Sen (1937). The actual amounts of salts in the standard nutrient in grams per pot were as follows:

NaNO_3 , 7.37; $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 2.04; K_2SO_4 , 1.50; $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$, 0.30; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.01.

Iron and manganese were also added. The nutrients were not applied in one dose as in previous experiments, but in three equal doses at fortnightly intervals, the first being given immediately after germination. Besides the standard set, five other nutrient combinations were used. In all these the amount of phosphate was reduced, and in three of them potassium also was proportionally reduced. Where sodium phosphate was reduced the requisite amount of sodium sulphate was added to bring the sodium level up to that of the standard. Sulphate was thus the only variable ion apart from those under investigation.

The standard nutrient series will be designated P_1K_1 , the letters referring to the variable elements in the experiment, while the numerals indicate the grade of *deficiency* of the particular nutrient. The suffix '2' indicates that one-third only of the complete amount of the nutrient considered was applied; '3' that one-ninth, and '5' that one-eighty-first, was given. The numbers are thus those of the terms of a geometric series having one as the first term and one-third as the factor. ' P_3K_1 ' therefore refers to a treatment in which all nutrients other than phosphorus were high, phosphorus being at one-ninth the standard level; similarly, in ' P_3K_3 ' both phosphorus and potassium were

reduced to one-ninth of their standard amounts, other elements being at their standard level. Using this terminology, the treatments used in the experiment, together with the number of replicate pots, were P₁K₁, 70; P₂K₂, 70; P₃K₁, 80; P₃K₃, 80; P₅K₁, 100; and P₅K₅, 25. Owing to the large number of pots required in any treatment to obtain complete data, only 25 were available for series P₅K₅; in this series therefore the data are fragmentary, samples being taken only over the period of maximal effect.

As in previous work, the experimental material consisted of the successive individual leaves on the main axis, and these were always sampled at the time of complete emergence. In practice this entailed a small amount of compromise, since the rate of leaf emergence of plants treated identically varies slightly, and it was necessary to judge when the average leaf of each treatment was just emerged. Between treatments there were very considerable differences in the rates of leaf emergence; these increased during the life history, so that while the tenth leaf of P₁K₁ was sampled on July 8, that of P₅K₁ was not ready until August 16, five and a half weeks later. The first leaf was not sampled, and the remainder were collected on the following twenty-six sampling dates:

TABLE I

Dates of Sampling of Successive Leaves in the Various Treatments

Leaf.	P ₁ K ₁ .	P ₂ K ₂ .	P ₃ K ₁ .	P ₃ K ₃ .	P ₅ K ₁ .	P ₅ K ₅ .
2	May 29	May 29	May 29	May 29	May 29	—
3	June 5	June 5	June 7	June 7	June 8	—
4	June 8	June 8	June 15	June 15	June 17	—
5	June 15	June 17	June 21	June 21	June 28	—
6	June 17	June 20	June 29	June 29	July 8	July 8
7	June 24	June 28	July 5	July 5	July 18	July 18
8	June 28	July 3	July 12	July 12	July 26	July 24
9	July 3	July 5	July 17	July 17	Aug. 7	Aug. 2
10	July 8	July 10	July 22	July 22	Aug. 16	Aug. 10
11	July 10	July 12	July 28	July 28	—	—

Usually 4–5 gm. of leaves were gathered at each sample to determine respiration rates. From every plant that had furnished a single newly emerged leaf, the previous leaf on the main axis was also at the same time collected; similar leaves were massed together for respiration determination. The respiration rate of each leaf from the third to the tenth in every treatment was thus determined on two occasions, namely, at the time of its complete expansion and also at the time of complete expansion of the succeeding leaf. In many cases the tips of the leaves from the very deficient series at their second time of sampling, or even a considerable portion of their distal ends, were shrivelled and dead. Dead parts were removed before weighing and determining respiration rates. Only in two or three instances was it necessary to remove the extreme tips of any leaves at the time of their emergence; these all belonged to series P₅K₁.

The method of determining the CO_2 evolved in respiration was identical with that adopted by Gregory and Sen (1937), and need not be repeated here. The thermostatically controlled box containing the leaf chambers was maintained where possible at 25°C ., but unfortunately several of the samples were taken on very hot days, so that the temperature of the laboratory rose well above this. In extreme cases the temperature in the leaf chambers rose to 27°C . Temperatures above 25°C . occurred on nine occasions (June 5, 7, and 8; July 3, 5, 8, 10, and 24, and August 7), and in these instances the observed respiration rates have been adjusted to 25°C ., assuming a Q_{10} of 2.

Respiration rates were usually determined over two successive periods of three hours each, after which the leaves were placed in a ventilated oven at 70°C ., spread out on perforated trays and dried rapidly. Those taken at the time of their emergence were supplemented by a further number of similar leaves which had been collected at the same time as the respiration samples and immediately dried. The total material, 6–10 gm. of leaves, after drying, was sealed while hot in bottles and stored for nitrogen analyses. They were subsequently analysed by Mr. H. Said¹ for the following fractions: total nitrogen, total crystalloid nitrogen, and amino nitrogen. The difference between the two former gives an estimate of protein nitrogen. The methods of extraction and analysis were identical with those described by Richards and Templeman (1936).

Finally, at the time of sampling, two further duplicate lots, of roughly 1 gm., were also taken for the determination of total and reducing sugars. The leaves were weighed and killed immediately by plunging into boiling alcohol. Subsequently they were extracted in alcohol and the extract transferred to water, alcohol being removed by evaporation under reduced pressure at 30°C . The reducing power before and after hydrolysis by invertase was determined without clearing by the method of van der Plank (1936). The total sugar estimations do not include fructosans.

EXPERIMENTAL RESULTS

General remarks.

The general symptoms of phosphorus and potassium deficiency have been described previously (Gregory and Richards, 1929, and particularly Richards and Templeman, 1936). The external symptoms developed in all the deficient series of this experiment, even when potassium was reduced proportionally to phosphorus, were those usually associated with phosphorus deficiency. As was shown by Verma (1935) it is necessary to reduce potassium to considerably lower levels than phosphorus in order to obtain a plant in which, judged by external symptoms, potassium and phosphorus are again balanced; if the ratio is decreased further, symptoms associated with potassium deficiency may be obtained. The transition from one type of plant to the other is sharp. The lower the general level of phosphorus and potassium nutrition

¹ These data were incorporated in a thesis for the D.I.C. degree in 1934.

the higher must be the P/K ratio in order to obtain balanced conditions between these elements. The present P₂K₂ series differed from the P₁K₁ in the direction of phosphorus deficiency, having reduced tillering rate and leaf size and a darker colour, with some tendency to anthocyanin formation. In the series P₃K₁ and P₃K₃ these symptoms were more marked, but plants from these two treatments were indistinguishable in appearance and also in their rates of development, so that all sampling dates for the two series were identical (Table I). The series P₅K₁ and P₅K₅ developed acute symptoms of phosphorus deficiency, grew slowly, remained very dwarf, and produced very few tillers. Throughout the main vegetative period they were indistinguishable, but on reaching the stage of stem elongation, when in some measure recovery from starvation symptoms sets in, differences became manifest. The recovery in the series P₅K₅ was more rapid and complete than in the P₅K₁, and by the time the tenth leaf emerged there was approximately a difference of one week in their developmental stage. Clearly at such low levels of phosphorus, excess of potassium and nitrogen is more detrimental than excess of nitrogen alone.

Except at the lowest phosphorus level, it was usual for the plants to produce eleven leaves on the main axis. In series P₁K₁ an occasional minute twelfth leaf was produced; for example, out of the twenty-three leaves taken at the last respiration sample, eighteen were last leaves while five were rather larger, with a very small twelfth leaf beyond. At the P₅ level it was usual for ten leaves only to be produced, but occasional variation to nine or eleven was found.

Leaf dry weight.

The data for the average dry weights of the individual leaves at expansion are given in Table II. In all series, with the possible exception of P₅K₅

TABLE II
Mean Dry Weight of Single Leaves at Expansion, in mg.

Leaf.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.
P ₁ K ₁	26.9	44.9	59.0	66.7	88.4	149.6	185.4	163.0	118.9	45.4
P ₂ K ₂	25.1	38.0	50.7	57.0	78.1	108.2	153.0	141.1	88.8	23.6
P ₃ K ₁	22.1	27.7	40.2	47.2	59.5	87.6	120.7	111.8	71.3	23.9
P ₃ K ₃	20.8	26.1	36.1	39.6	61.8	84.3	114.7	108.0	69.8	21.8
P ₅ K ₁	21.4	23.3	25.5	29.1	36.6	42.8	50.4	44.6	39.2	—
P ₅ K ₅	—	—	—	—	36.5	48.7	51.4	54.9	31.9	—

maximum weight is attained by the eighth leaf. The rise in weight is fairly gradual up to leaf 5 or 6, then rapid to 8. A sharp decline occurs after leaf 8. The general course is unaffected by the wide differences in nutrient treatment though the actual weights are very different in the various series. Differences due to phosphorus supply have appeared as early as leaf 2, and are large in leaf 3, where the weight in series P₁K₁ is almost double that of P₅K₁. Maximum differences occur in leaves 7–9, P₁K₁ giving a leaf some three and

a half times as heavy as P₅K₁. In spite of the absence of easily observable differences between plants from series P₃K₁ and P₃K₃, the mean of a large number of replicate leaves discloses that there is a slight difference in size, P₃K₁ being throughout the series consistently rather heavier than P₃K₃. This difference is apparently real, having a statistical probability less than $P = 0.01$. At the P₃ level therefore, reduction in potassium supply to a level proportional to that of the phosphorus leads to a further slight but real reduction in individual leaf size. At the P₅ level there is no statistical evidence of a real effect ascribable to difference in potash supply, but the small difference found is in favour of P₅K₅, which is perhaps consistent with the better condition and faster development of plants in this series.

Water content.

The water content data at both stages are presented in Tables III and IV. Since treatment differences are comparatively slight, while at the same time a complex relationship to manuring exists, it is essential to present a statistical

TABLE III
Water Content (% Dry Weight) at Time of Complete Expansion

Leaf.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.
P ₁ K ₁	526	532	490	475	470	431	391	318	272	271
P ₂ K ₂	528	519	483	438	498	433	386	312	275	261
P ₃ K ₁	584	603	477	490	559	496	323	276	284	245
P ₃ K ₃	551	576	497	517	525	473	322	273	283	226
P ₅ K ₁	566	613	527	506	469	381	460	412	379	—
P ₅ K ₅	—	—	—	—	450	323	370	314	305	—

TABLE IV
Water Content (% Dry Weight) at Complete Expansion of Succeeding Leaf

Leaf.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.
P ₁ K ₁	—	472	478	472	499	440	386	314	284	—
P ₂ K ₂	—	471	443	447	447	444	356	313	246	—
P ₃ K ₁	—	506	456	491	520	420	300	274	297	—
P ₃ K ₃	—	467	447	454	543	396	324	289	259	—
P ₅ K ₁	—	497	469	494	415	456	456	406	—	—
P ₅ K ₅	—	—	—	491	392	402	378	370	—	—

analysis. The change in water content with leaf number is in all series similar to that observed in the fully manured series of previous experiments (Gregory and Richards, 1929; Richards, 1932). The largest effect of phosphorus deficiency is found in series P₅K₁, where at the time of full emergence all leaves, with the exception of 6 and 7, show higher moisture contents than corresponding leaves from series P₁K₁. Analysis of the variance of the nine pairs of observations into that directly ascribable to leaf number, that to phosphorus supply, and the remainder, gives a 'z' value for the phosphorus effect of 1.012 (5% point 0.836; 1% point 1.211). Hence there is no reason to doubt that

extreme phosphorus starvation leads to a slight but real increase in water content; this difference is still noticeable at the time of the second determination on the leaves. It may perhaps be emphasized that leaf 10 in series P₁K₁ was sampled on the same day as leaf 6 of P₅K₁, and owing to the fact that water content falls rapidly in later leaves, the difference between the uppermost leaves from the two series on that particular day was very much greater than that between either leaf 6 or leaf 10 of the two treatments. Hence by comparing samples of leaves taken at the same time from plants differently treated regardless of the real growth stage attained, spurious results may easily be obtained.

The change in water content brought about by difference in phosphorus supply, established above, is diametrically opposed to that found in previous work (Gregory and Richards, 1929), in which starvation to one-fifth the normal level produced a slight but consistent fall in water content. A precisely similar statistical analysis of those data to that adopted above, using the first determination on each leaf, gives a 'z' value of 1.054 (5% point 0.861; 1% point 1.253). It appears then that the direction of the change induced by phosphorus deficiency depends on the degree of severity: a reduction in water content follows moderate, an increase extreme, starvation. The present P₃K₁ series with one-ninth the standard amount of phosphorus lies between these two levels, and moreover near a critical level, for the slight average positive deviation in water content from the P₁K₁ value is quite without statistical significance.

There is no doubt therefore that a complex relationship exists between leaf water content and phosphorus supply, reduction or increase in succulence being obtained according to the degree of starvation, and even at some levels according to the period in life history. Similarly a complex interaction is found between potassium and phosphorus supply. It has been shown (Richards and Templeman, 1936; Gregory and Sen, 1937) that with the type of nutrient solution used (rich in sodium),¹ water content increases progressively with decreasing supplies of potassium, when all other nutrients are maintained at high level. This increase of water content with decreasing potassium supply does not hold at lower phosphorus levels. For example, at the P₃ level a reduction in potassium from K₁ to K₃ leads to no appreciable change in water content, the mean value over ten leaves being 2.2 per cent. lower in series P₃K₃ than P₃K₁, and 1.6 per cent. higher in series P₃K₃ than in P₁K₁. These differences do not approach significance. At the P₅ level, on the contrary, a lowering of potassium supply from K₁ to K₅ leads to a pronounced *lowering* of water content in the later leaves, where alone data are available. The fall is clearly real, and occurs at both stages of every leaf observed. Even using alone the five pairs of observations at the time of emergence, a 'z' value of 1.577 (1% point 1.527) is obtained for the potassium effect,

¹ Later work has emphasized the importance of the kations in the culture solution. A preliminary resumé of these developments has been made by Gregory (1937).

while yet higher significances may be obtained by including leaf age as a third variate in the analysis. The change in water content induced by reduction of phosphorus is thus approximately eliminated by a proportional reduction in potassium supply, and vice versa; the effects of the two nutrients are in no way additive. Extreme reduction in phosphorus (nitrogen and potassium at high level) leads to a pronounced increase in water content, while previous work has shown that extreme reduction in potassium (nitrogen and phosphorus at high level) produces yet greater succulence; simultaneous reduction in both phosphorus and potassium (nitrogen at high level), however, causes very little change from normal. The two nutrients, phosphorus and potassium, do not act independently; but an important factor is undoubtedly their relative concentrations in the culture solution. Balanced levels of the two nutrients result in almost uniform water content regardless of their absolute level. Thus the mean values for leaves 2-11 are as follows: P1K1, 418; P2K2, 413; P3K3, 424; while the means for leaves 6-10 are: P1K1, 376; P2K2, 381; P3K3, 375; P5K5, 352.¹ At moderate deficiency levels, change of the P/K ratio from the balanced value results in a change of water content in the same sense; while in extreme deficiency, deviation of the ratio from the balanced value in either direction leads to considerably increased water content.

Respiration rate.

In general the rate of CO₂ evolution of every batch of leaves was determined in each of two successive periods of three hours. To economize space these results are not presented in full. It may, however, be observed that the mean value of the respiration rate in the second period is 84 per cent. of that in the first, that the fall is greatest in the P1K1 series, and that in all treatments it is greater in early than in later leaves; the results thus completely confirm those of Gregory and Sen (1937). There is strong evidence that the rate of fall is closely related to the actual respiration rate, i.e. respiration in a leaf with a high initial rate tends to fall more rapidly than in one with a low initial rate. This agrees with the hypothesis that the substrate is used more rapidly in the former than the latter, but no definite evidence is found that the rate of fall is closely related to the actual sugar content of the leaf.

Yemm (1935) states that in excised leaves of barley, respiration rate rises during approximately the first twelve hours. The present experiments, and those of Gregory and Sen, demonstrate that this is not a general phenomenon, nor indeed a usual one. The leaves used by Yemm were among the last produced before the ear, and they were sampled not at the stage immediately following expansion but considerably later. With the possible exception of

¹ The fact that this figure is rather lower than the others is not likely to be significant, since it depends on one determination only, that of leaf 7. This particular leaf was the only one in the series from which it was necessary to remove dead and shrivelled tips; hence the distal portions probably had considerably reduced water content. Omitting this leaf the comparison of the remaining four gives: P1K1, 363; P2K2, 368; P3K3, 351; P5K5, 360.

leaves from extreme nitrogen-deficient treatments, such late leaves advancing towards senescence would appear to be the only ones in which a rising rate may be found, and even so the rise is slight; a rapid fall is more frequent. A further possible explanation of the difference is that while Yemm's leaves were collected in the late afternoon, those of the present experiment were sampled some five hours earlier, at approximately 11 a.m. The consequent difference in carbohydrate content, and a possible water deficit difference, may be factors concerned. Even the amount of handling undergone by leaves would appear to influence the early course of respiration after excision (Audus 1935, Godwin 1935).

TABLE V

Rate of Respiration (mg. CO₂ per gm. Dry Weight per Hour) at Time of Complete Expansion (25° C.)

Leaf.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.
P1K1	8.16	6.31	6.05	6.23	5.72	4.81	5.39	4.14	4.21	3.29
P2K2	7.96	5.22	6.11	5.14	5.90	4.60	4.25	3.72	3.67	4.77
P3K1	6.07	5.49	4.95	4.52	4.72	3.87	3.72	3.85	3.93	3.68
P3K3	6.05	5.29	5.61	4.84	5.16	4.66	3.89	3.96	4.20	4.16
P5K1	5.56	4.73	4.17	4.08	3.45	3.55	3.09	2.67	3.03	—
P5K5	—	—	—	—	3.56	3.16	3.12	3.10	—	—

TABLE VI

Rate of Respiration (mg. CO₂ per gm. Dry Weight per Hour) at Time of Complete Expansion of Succeeding Leaf (25° C.)

Leaf.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.
P1K1	—	7.55	7.22	6.40	4.74	5.78	4.16	3.85	3.12	—
P2K2	—	5.88	5.43	5.36	4.56	3.90	4.44	3.17	4.43	—
P3K1	—	4.99	4.09	4.43	3.55	3.61	3.41	3.33	2.90	—
P3K3	—	4.79	4.13	4.26	4.26	3.44	3.75	3.61	3.09	—
P5K1	—	3.07	3.45	3.37	3.33	2.71	2.13	2.51	—	—
P5K5	—	—	—	3.09	3.33	2.59	2.97	2.59	—	—

The mean respiration rates over the six-hour period are presented in Tables V and VI and in Figs. 1 and 2, Table V and Fig. 1 giving the rates in terms of leaf dry weight at the time of complete expansion, and Table VI and Fig. 2 the rates at the time of expansion of the next succeeding leaf on the main axis. The curves in Fig. 1 are nearly parallel and widely spaced. Maximum respiration rate is recorded in the second leaf, later successive leaves in all treatments showing a steady decline to leaf 8 or 9, with a tendency in some at least to rise slightly again in the last leaf or two. The effect of phosphorus deficiency is already pronounced at the second leaf, P1 having the highest rate and P5 the lowest. These differences are maintained throughout the leaf series. Analysis of the total variance of the twenty-six degrees of freedom obtained from leaves 2-10 of series P1K1, P3K1, and P5K1 into that representing differences in phosphorus level (two degrees), that due to differences among the various leaves (eight degrees), and the remainder on which the estimate of error is

based (sixteen degrees), gives a 'z' value of 2.069 (1% point 0.914) for the phosphorus effect and one of 1.557 (1% point 0.679) for the effect of leaf number. The differences between treatment means are as follows:

$$P_1K_1 - P_3K_1 = 1.10 \pm 0.167$$

$$P_3K_1 - P_5K_1 = 0.75 \pm 0.167.$$

There is therefore no question of the reality of the phosphorus effect over the whole range of levels considered, and the absence of effect reported in a previous paper (Gregory and Richards, 1929) was clearly due to the slight

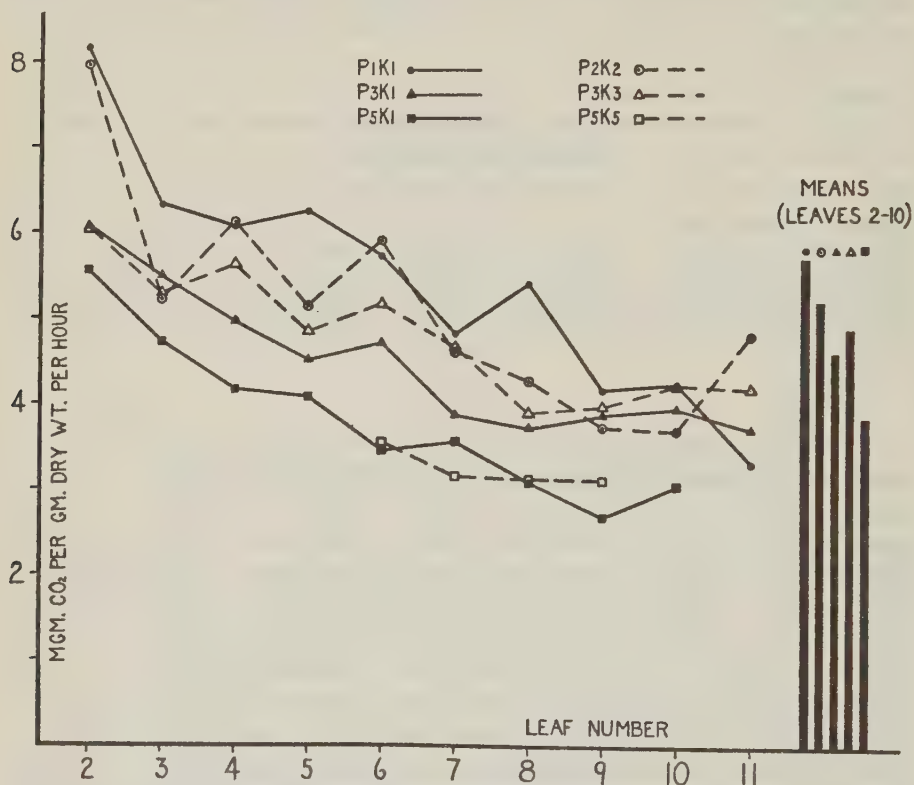


FIG. 1. Respiration rates of the various series for successive leaves at emergence. Histograms give treatment means. Nomenclature: P, phosphorus; K, potassium. Suffixes: 1, full supply; 2, $\frac{1}{2}$ supply; 3, $\frac{1}{3}$ supply; 5, $\frac{1}{5}$ supply.

degree of deficiency investigated, to the fact that all the nutrients were applied at the beginning, and to the larger sampling error involved in the use of very small amounts of leaf material.

The eight degrees of freedom representing leaf number differences may be further subdivided into one representing the linear regression of respiration rate on leaf number, and seven representing deviations from that regression. A comparison of these two variances gives a 'z' value of 2.115 (1% point 1.253) for the regression, while that due to the seven degrees of freedom

representing deviations from the regression is not significantly greater than that of the sixteen ascribed to general error ($'z' = 0.433$; 5% point 0.492). On the average therefore a uniform decrease in respiration rate with leaf number gives a fairly adequate representation of the data. But it must be

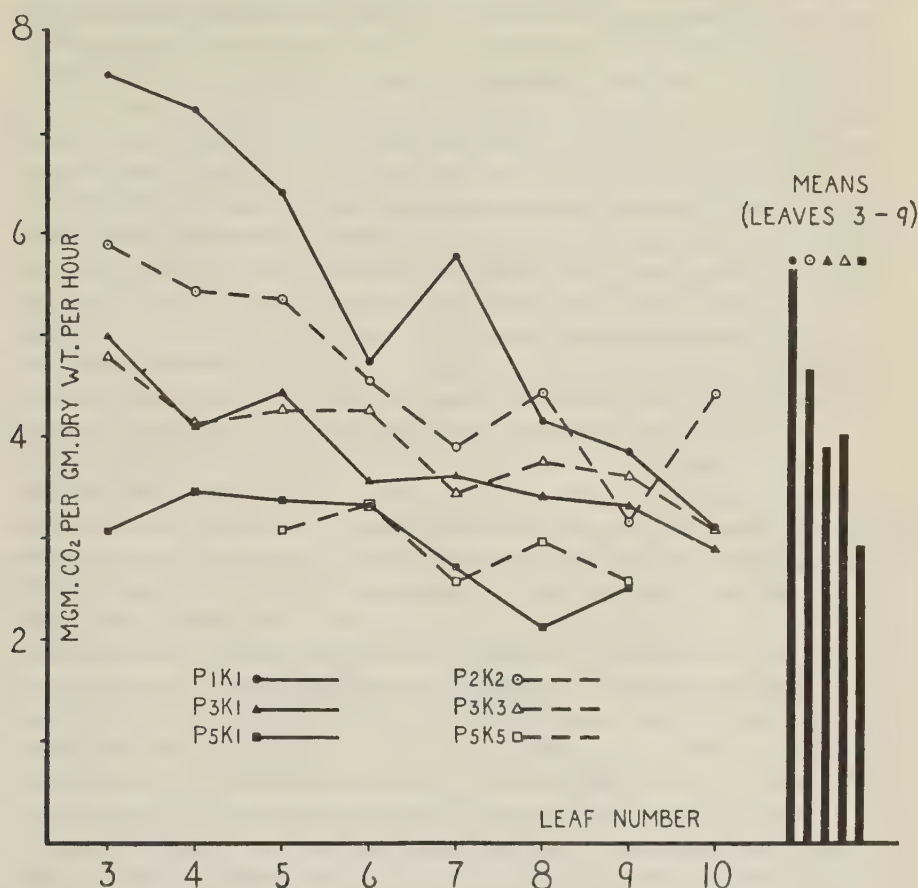


FIG. 2. Respiration rates of the various series for successive leaves at the time of emergence of the next succeeding leaf. Histograms give treatment means. Nomenclature: P, phosphorus; K, potassium. Suffixes: 1, full supply; 2, $\frac{1}{2}$ supply; 3, $\frac{1}{3}$ supply; 5, $\frac{1}{5}$ supply.

remembered that replicate determinations were not made, and therefore that there is confusion in the above analysis between variance caused by real interaction between phosphorus level and leaf number and that strictly attributable to error. This may be investigated by determining the individual regressions of the three treatment curves. All three prove to have highly significant linear regressions, but when a parabola is fitted to the data the extra goodness of fit over that given by the straight line is negligible in series P1K1 but highly significant in series P3K1 ($'z' = 1.380$; 1% point 1.253) and in P5K1 ($'z' = 1.159$; 5% point 0.895; 1% point 1.310). In these two deficient

series, therefore, the fall in respiration rate with leaf number is considerably greater among early leaves than later, and the tendency of the observed rates in the last leaves to show a secondary rise is probably real, at least in series P₃K₁.

It has been shown previously that at high levels of nitrogen and phosphorus, reduction of potassium supply leads to a considerable increase in respiration rate. The present data demonstrate that when phosphorus is maintained at one-ninth its standard level a corresponding reduction in potassium is again reflected in increased respiration rate. The increase of P₃K₃ over P₃K₁ is not nearly so great as that previously found at high phosphorus levels, the mean increase being less than 7 per cent. and the maximum three times as great. But the difference is found consistently after the third leaf, and an analysis of the variance of leaves 2-11 in the two treatments shows that it must be considered real: the 'z' value representing the potassium effect is 1.147, the 5% point being 0.816, and the 1% point 1.179. On the other hand, the four comparisons available at the P₅ level appear to indicate that here a proportional reduction in potassium to that of phosphorus is without appreciable effect on respiration rate; the difference between the treatment means is only 1.4 per cent. The corresponding difference at the time of expansion of the next succeeding leaf is 3.7 per cent. in favour of P₅K₅; this difference does not approach significance, and if real is likely only to express the fact that leaves of the series P₅K₁ age earlier than those of the P₅K₅ series. It was found by Gregory and Sen that very low levels of nitrogen mask the potassium effect completely; it appears then that nitrogen and phosphorus are alike in that deficiency of either nutrient leads to considerable reduction in respiration rate, and that extreme deficiency of either completely eliminates the potassium effect. These two nutrients have many other effects in common opposed to those of potassium (cf. Richards and Templeman, 1936).

When phosphorus and potassium are simultaneously reduced respiration rate likewise falls. An analysis of the respiration values of leaves 2-11 in series P₁K₁, P₂K₂, and P₃K₃ gives a 'z' value of only 0.620 for the treatment effect, the 5% point being 0.634; but the standard error reveals that the greatest difference, that between P₁K₁ and P₃K₃, is likely to be real, i.e. 0.649 ± 0.247 . In leaf 11 the values are somewhat erratic and necessarily more uncertain than the others, and if this leaf be omitted from the analysis 'z' reaches a value of 0.979 (1% point 0.914). It is now found that the difference between the means of P₁K₁ and P₂K₂ reaches significance level (0.445 ± 0.197), though that between P₂K₂ and P₃K₃ is still only 0.291 ± 0.197 . It has been shown that P₃K₃ has a significantly greater rate than P₃K₁, while there is little difference between P₅K₅ and P₅K₁. It may therefore be concluded that respiration rate falls on simultaneously reducing phosphorus and potassium below the level in P₁K₁, but that the rate of fall at moderate levels is not so great as when phosphorus alone is reduced; as starvation becomes acute simultaneous variation of phosphorus and potassium leads to more rapid change in respiration rate than does variation of phosphorus alone.

It may be of interest to point out that if the respiration values of the individual leaves are plotted against time from germination (date of sampling) instead of against leaf number as in Fig. 1, the manurial differences found in the early stages of life history rapidly diminish. Thus between July 8 and 12 were sampled leaves 10 and 11 of P1K1 and P2K2, leaf 8 of P3K1 and P3K3, and leaf 6 of P5K1 and P5K5. At this time therefore these particular leaves were the 'dominant' leaves, i.e. the uppermost expanded leaves on the main axis. Their respiration rates were as follows: P1K1, mean, 3.75; P2K2, mean, 4.22; P3K1, 3.72; P3K3, 3.89; P5K1, 3.45; and P5K5, 3.56. The value for leaf 11 of P2K2 is clearly too high, the rate of the tenth leaf on July 10 being 3.67. It appears then that had respiration rates of the youngest leaves from all series been compared at certain specified times, regardless of the stage of development reached, the conclusions drawn concerning the effect of phosphorus would have been very different from those given here. It may be stressed therefore that when investigating the effect on some process of a variable factor which affects the developmental rate of the plant, a comparison between data obtained from the various treatments by sampling all at the same time may give an entirely false representation of the real effect of that factor (cf. Richards, 1934).

Table VI and Fig. 2 give the respiration rates of the leaves at the time of their second sampling, i.e. at the time of complete emergence of the next succeeding leaf. The results are very similar to those already presented and will not therefore be described in detail. The same types of difference between treatments and between successive leaves are found. The curves for series P1K1, P3K1, and P5K1 are more widely spaced than previously, and there is again strong indication of a potassium effect at the P3 level after leaf 5, but none appears at the P5 level. At these lower levels of nutrition the treatment curves are nearly parallel to and lower than the corresponding curves in Fig. 1. It may easily be demonstrated that this fall in respiration rate with leaf age is real in all four series at the P3 and P5 levels, while in series P1K1 it is only in later leaves that a consistent fall in respiration with leaf age is found—whereas a possible rise occurs in the earlier leaves. The observed difference in behaviour with age between leaves from the P1K1 series and those from deficient series may be largely spurious; for under deficiency the absolute length of life of any particular leaf is considerably less than under conditions of complete nutrition, while the rate of leaf production is much reduced, so that the absolute difference in time between the two observations in any leaf is considerably greater in the deficient series. In consequence, the second determination on a leaf in the P1K1 series is made before senescence is advanced, while under starvation it is made at a later stage of the leaf, when in fact a considerable portion of the distal end has already died. The manurial differences at this second determination are therefore confounded with age differences, and the latter act in such a way as to exaggerate the direct treatment effects.

By way of summary, a general picture of the phosphorus-potassium

interaction in respiration rate at the time of leaf expansion may be constructed. For this the mean treatment rates between leaves 2 and 10 have been used, that of series P₅K₅ being assumed identical with P₅K₁; for the later leaves provide no evidence of respiration differences between the two series, and it is unlikely that the earlier would do so, so that the treatment difference between these series must be very slight. Two extra points may be obtained from the results of Gregory and Sen's experiment, conducted under identical conditions but in a previous season. The series N₁K₃ and N₁K₅ of Gregory and Sen occupy positions P₁K₃ and P₁K₅ in the present scheme, and to these is applied a small correction for seasonal difference, proportional to the relative values of respiration found in series P₁K₁ and the identical treatment N₁K₁ of the previous year. There are thus, in all, eight treatment points, three of which represent a phosphorus series, three a potassium, and four a series in which both vary proportionally. Stating phosphorus and potassium levels in the conventional terms used here (P or K = 1, 2, 3, 4, or 5) a parabola may be fitted to both variables to represent their general relationship, and also a linear term to the ratio of phosphorus to potassium in order to give expression to the interaction. The derived equation is:

$$\text{Respiration rate} = 5.521 - 1.668 P + 0.1256 P^2 + 1.472 K - 0.1763 K^2 \\ + 0.4593 P/K.$$

The regression has a multiple correlation coefficient of 0.991. Contours of equal respiration rate over the whole treatment range, derived from the equation, are shown in Fig. 3, and all important features of the interaction, established above, are there represented. The very different relation of the two nutrients to respiration is well brought out. As phosphorus decreases (P₁K₁–P₅K₁) respiration falls, rapidly in the early stages of deficiency, more slowly later; while as potassium decreases (P₁K₁–P₁K₅) respiration rises to a maximum at about K₄ level, falling slightly to K₅ level. At yet lower potassium levels (Richards, 1932) the decline in respiration rate due to carbohydrate limitation is known to continue, as would also occur in the diagram if this were extended beyond the K₅ side. At the P₃ level changes in respiration rate with potassium are much slighter, but of the same form, while at the P₅ level they are practically non-existent; in fact the surface in this region is nearly flat, and if the diagram were continued beyond the P₅ side the next contour line, 3.5, would not be reached over the whole potassium range, for the surface again rises. Along the diagonal series P₁K₁ to P₅K₅ the rate of fall increases with the degree of deficiency, being at first considerably less than along the K₁ side, but later greater. Relative to phosphorus supply the rates of change in these two directions are approximately equal at the P₃ level. It may also be observed that the line joining combinations with the same respiration rate as series P₁K₁ passes very close to P₂K₃ and thereafter runs nearly parallel to the potassium axis. This line is of the same form in the diagram as that found by Verma (cf. p. 494) connecting treatments which

produce plants having external characteristics similar to those of P_1K_1 , differing only in size, and showing no marked external symptoms of either phosphorus or potassium deficiency. It appears that such balanced combinations produce little deviation from the normal in respiration rate, but that a

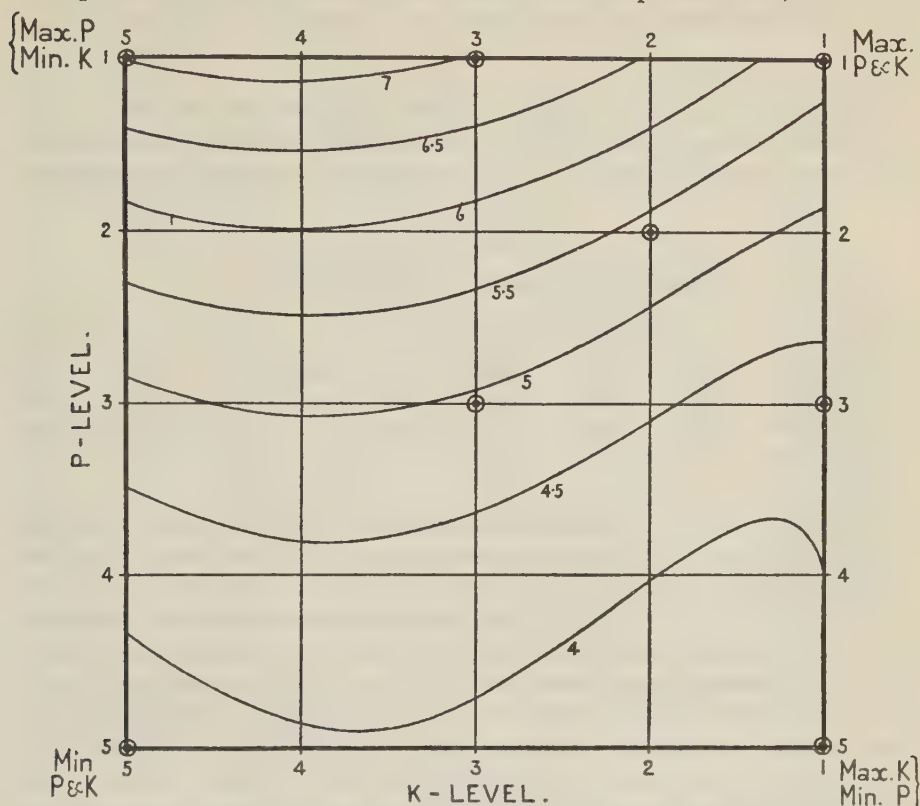


FIG. 3. General interaction of phosphorus and potassium supply as affecting leaf respiration rate. Contours show mean respiration rate of leaves 2-10 (mgm. CO_2 per hour per gm. dry weight at $25^\circ C.$). P_1 - P_5 , diminishing phosphorus; K_1 - K_5 , diminishing potassium. Circles indicate experimental combinations from which regression is derived.

change from such a balanced condition to any other producing external symptoms of potassium deficiency involves increased respiration, while a change leading to symptoms associated with phosphorus deficiency similarly involves a lowering of respiration rate. Finally, while the diagram represents adequately the average treatment effects over all leaves, the early leaves, and also the latest, have relatively smaller respiration differences, while those produced in the middle of the vegetative period have more pronounced differences, than are indicated by the diagram. The interactions with leaf number are relatively unimportant, so that the diagram represents closely the respiration rate at all stages of growth except that the absolute magnitudes will be different. Apart from these changes in magnitude, the most important change with

leaf number concerns the position of the maximum along the P₁ side. In the first and last leaves this is apparently shifted beyond the K₅ limit of the diagram, and rising rates are found over the whole K₁–K₅ range; while at the stage when minimum internal carbohydrate levels occur the ridge presumably shifts in the opposite direction (Gregory and Sen, 1937; Richards, 1932).

Nitrogenous compounds.

To economize space the remaining experimental data are presented briefly.

(a) *Total nitrogen* (Table VII). It was found previously (Richards and Templeman, 1936) that with a moderate degree of phosphorus deficiency,

TABLE VII
Total Nitrogen Content (% Dry Weight)

Leaf.	3.	4.	5.	6.	7.	8.	9.	10.	11.
P ₁ K ₁	5.39	4.65	4.61	4.94	3.64	3.04	3.12	3.21	3.47
P ₂ K ₂	5.18	4.23	3.84	3.92	3.68	3.26	3.46	3.55	3.17
P ₃ K ₁	4.51	4.42	3.67	3.49	3.60	3.07	2.86	3.46	4.22
P ₃ K ₃	4.66	3.83	5.37	5.50	3.79	3.06	3.33	3.87	4.48
P ₅ K ₁	4.22	4.18	4.19	4.01	3.10	3.94	4.47	4.03	—
P ₅ K ₅	—	—	—	4.71	2.89	3.48	3.77	4.11	—

approximately level P₃, total nitrogen content is reduced. The present results confirm this, but at the P₅ level the average content of the leaves is again as high as in series P₁K₁, the mean values for leaves 3–10 being: P₁K₁, 4.08; P₃K₁, 3.64; P₅K₁, 4.02. It may be shown that on the average the minimum at P₃ is real, but there are large interactions. Thus the excess nitrogen content of series P₁K₁ over that in both series P₃K₁ and P₅K₁ decreases significantly with leaf number, while that of series P₃K₁ over P₅K₁ is at first positive and later negative, but the trend does not quite attain the significance level. The leaf means from the three series also fall significantly with leaf number. The main effects of phosphorus deficiency may therefore be summarized as follows: (1) in early leaves progressive P deficiency leads to progressive reduction in nitrogen content; (2) at the P₃ level the general fall with leaf number is not so pronounced as in series P₁K₁, so that later leaves of the two series P₃K₁ and P₁K₁ show no appreciable difference in nitrogen content; (3) at the P₅ level the change with leaf number is largely eliminated and although the earlier leaves have lower nitrogen contents than those from higher phosphorus levels, the later leaves have the highest nitrogen content of any.

No significant difference can be demonstrated between the nitrogen contents of series P₃K₁ and P₃K₃, or of P₅K₁ and P₅K₅, but it is probable that P₃K₃ contains rather more than P₃K₁. The values for leaves 5 and 6 are unusually high in P₃K₃, and lead to a large estimate of error.

(b) *Protein nitrogen* (Table VIII, Fig. 4). A considerable, progressive, and very highly significant decrease of protein nitrogen with phosphorus deficiency is found throughout the main vegetative period between series P₁K₁, P₃K₁, and

P₅K₁, but from leaf 8 onwards the differences are smaller and here it appears that minimum contents are found at level P₃. An interaction evidently obtains, somewhat similar to that in total nitrogen, but to a lesser extent.

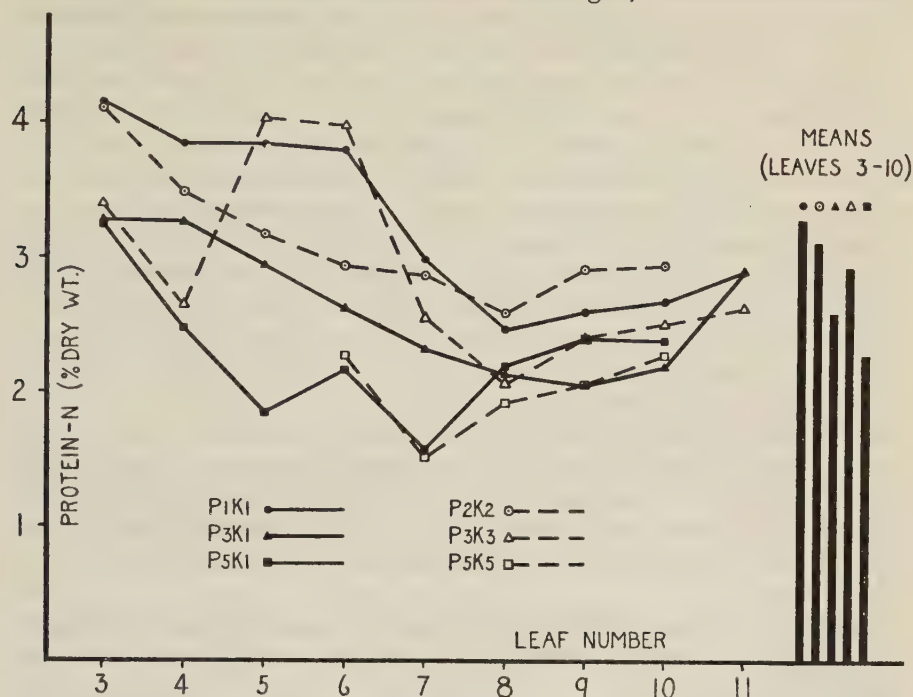


FIG. 4. Protein nitrogen contents of successive leaves at emergence. Histograms give treatment means. Nomenclature: P, phosphorus; K, potassium. Suffixes: 1, full supply; 2, $\frac{1}{3}$ supply; 3, $\frac{1}{6}$ supply; 5, $\frac{1}{32}$ supply.

TABLE VIII

Protein Nitrogen Content (% Dry Weight)

Leaf.	3.	4.	5.	6.	7.	8.	9.	10.	11.
P ₁ K ₁	4.16	3.85	3.85	3.81	3.00	2.48	2.61	2.69	2.92
P ₂ K ₂	4.11	3.49	3.18	2.95	2.88	2.60	2.93	2.96	—
P ₃ K ₁	3.28	3.27	2.95	2.63	2.33	2.14	2.06	2.20	2.93
P ₃ K ₃	3.40	2.65	4.04	3.99	2.56	2.07	2.41	2.52	2.64
P ₅ K ₁	3.26	2.48	1.85	2.17	1.58	2.20	2.41	2.40	—
P ₅ K ₅	—	—	—	2.28	1.52	1.93	2.06	2.29	—

Both the linear and quadratic regressions on leaf number are highly significant, hence the rapid drop in protein content between successive early leaves is not maintained throughout life history, but may be replaced by a rise. These relations are similar to those already found for respiration rate.

The mean difference in protein content between series P₃K₁ and P₃K₃ is 10 per cent., and is in favour of P₃K₃. This cannot be demonstrated to be real, mainly because the very high values in leaves 5 and 6 of P₃K₃ make the

estimate of error large, just as with total nitrogen. Nevertheless, it is probable that a real effect is indicated and that considerable interaction exists; for any deviations from the normal induced by simultaneous reduction of phosphorus and potassium to the level P₂K₂ may be expected to reappear on reduction to the level P₃K₃, the effects probably being exaggerated and occurring earlier in the life-cycle. Now the departures from the normal in the successive leaves of P₂K₂ form a fairly regular series, progressive relative reduction in protein being found to leaf 6, followed by gradual recovery, so that the last three leaves have super-normal contents. This course is repeated in an exaggerated form in series P₃K₃ between leaves 3 and 6, relative protein content subsequently declining. Again, the irregular course of the curve for series P₃K₃ is closely and inversely simulated by the corresponding curve in reducing sugar content, derived from independent samples, a correlation which will receive more attention later. It is unlikely therefore that the error in this series is considerably larger than in others, as would appear at first sight, and that in fact a large interaction effect exists.

As between series P₅K₁ and P₅K₅ it is again impossible to demonstrate a potassium effect, but the actual difference found is in favour of series P₅K₁. The general course in these two series is interesting, since the consistent rise from leaf 7 to 10 may readily be shown to be real; there is definite evidence therefore that at this phosphorus level a real recovery in protein content occurs in later leaves.

(c) *Amino nitrogen* (Table IX, Fig. 5). Decreasing phosphorus supply leads to rapidly increasing amino-nitrogen contents over the whole treatment

TABLE IX
Amino Nitrogen Content (% Dry Weight)

Leaf.	3.	4.	5.	6.	7.	8.	9.	10.	11.
P ₁ K ₁	0.367	0.419	0.496	0.307	0.234	0.220	0.196	0.149	0.152
P ₂ K ₂	0.342	0.375	0.466	0.394	0.343	0.323	0.239	0.199	—
P ₃ K ₁	0.453	0.515	0.609	0.435	0.510	0.418	0.346	0.572	0.555
P ₃ K ₃	0.609	0.634	0.765	0.541	0.605	0.478	0.437	0.642	0.724
P ₅ K ₁	0.472	0.872	0.962	0.796	0.626	0.707	0.806	0.786	—
P ₅ K ₅	—	—	—	1.033	0.602	0.738	0.774	0.802	—

range, the rise from series P₃K₁ to P₅K₁ being considerably greater than that from series P₁K₁ to P₃K₁. Interactions with leaf number exist: thus the excess amino content of either series P₃K₁ or P₅K₁ over P₁K₁ increases very significantly with leaf number, showing that the effect of phosphorus deficiency is in general progressive throughout the life-cycle. In series P₁K₁ the general decline with leaf number is also highly significant. These results completely confirm those found previously (Richards and Templeman, 1936).

The series P₃K₁ and P₃K₃ are remarkable in that all fluctuations in one are accurately reproduced in the other, and the curves are parallel, that for P₃K₃ being always above. These particular treatments were sampled on the same days throughout, and it appears that the minor fluctuations found are mainly

real, probably representing weather or other conditions common to the two. As a consequence, the estimate of error is extremely low and it is possible to demonstrate not only that the potassium difference is very highly significant, but that the maximum at leaf 5 and also the minimum at leaf 9 are real. A

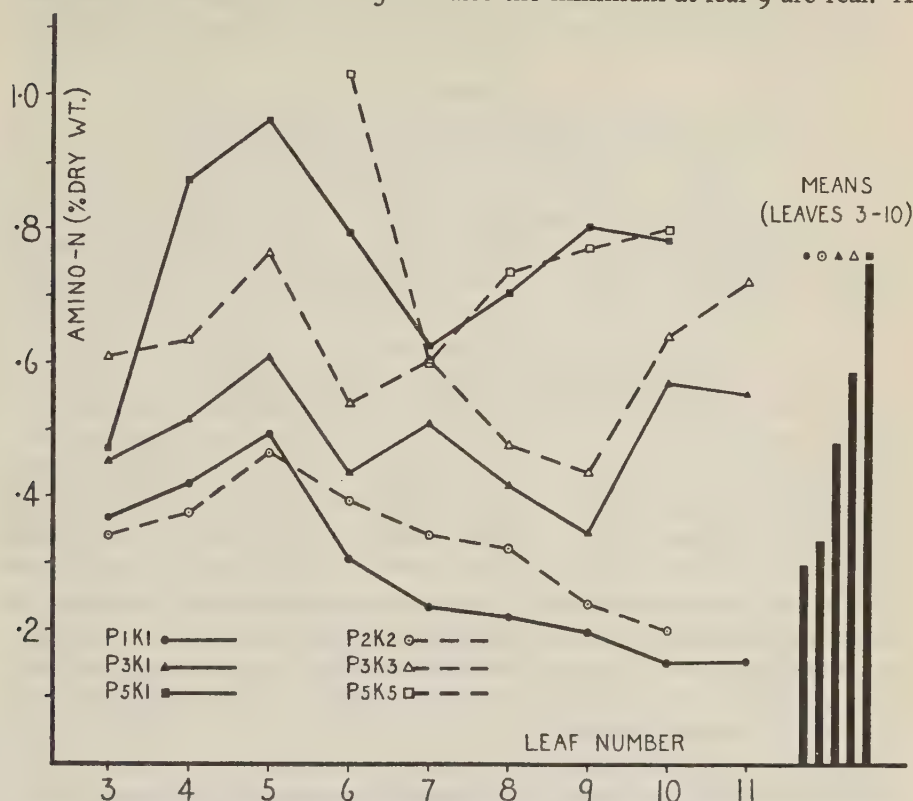


FIG. 5. Amino nitrogen contents of successive leaves at emergence. Histograms give treatment means. Nomenclature: P, phosphorus; K, potassium. Suffixes: 1, full supply; 2, $\frac{1}{2}$ supply; 3, $\frac{1}{3}$ supply; 5, $\frac{1}{5}$ supply.

similar maximum at leaf 5 is found in all other treatments. Under moderate phosphorus deficiency, therefore, amino content increases in the early leaves, declines to a minimum and again rises sharply in the last leaf or two. This secondary rise to a very high level was observed in previous work. A similar but more exaggerated course is found at the P5 level, but here there is little evidence that a corresponding reduction in potassium leads to any change in amino content, agreement between the two treatments being very close. The highly significant increase brought about by reduction of potassium at the P3 level amounts to 23 per cent.; this is not a large effect, for a similar reduction of potassium at the P1 level has been shown (Richards and Templeman, 1936; Gregory and Sen, 1937) to produce increases of amino nitrogen up to and exceeding 100 per cent.

Carbohydrates.

Copper reducing-power was estimated before and after hydrolysis by invertase. Reduction before hydrolysis is taken as the measure of free hexoses, and that after hydrolysis as the measure of total sugar, the difference giving the estimate of sucrose. In Tables X and XI are presented the contents of sucrose

TABLE X
Sucrose Content as Equivalent Hexose (% Dry Weight)

Leaf.	3.	4.	5.	6.	7.	8.	9.	10.	11.
P ₁ K ₁	12.3	16.2	13.8	11.3	12.0	14.4	10.0	10.3	5.3
P ₂ K ₂	13.4	16.1	13.2	12.9	11.4	11.8	12.7	5.1	8.7
P ₃ K ₁	10.9	14.0	8.1	16.5	6.9	10.0	9.0	8.4	5.5
P ₃ K ₃	8.2	12.9	11.2	10.8	11.3	11.3	13.0	8.6	6.6
P ₅ K ₁	6.8	5.5	9.8	11.4	12.7	12.1	7.2	5.7	—

TABLE XI
Reducing Sugar Content (% Dry Weight)

Leaf.	3.	4.	5.	6.	7.	8.	9.	10.	11.
P ₁ K ₁	0.91	1.23	1.33	1.10	1.65	1.45	1.40	1.67	1.52
P ₂ K ₂	1.10	1.58	1.34	1.47	1.56	1.62	1.59	1.08	1.49
P ₃ K ₁	1.31	2.17	1.74	1.97	1.01	1.21	1.72	1.71	1.16
P ₃ K ₃	1.05	3.33	1.61	1.62	1.80	2.38	1.72	1.41	1.17
P ₅ K ₁	1.49	1.50	2.16	1.59	1.98	1.86	0.90	0.91	—

and reducing sugar respectively in terms of dry weight, and by addition the content of total sugar may be obtained. The observed differences are generally not so great as with the nitrogen fractions, but there are considerable interactions between leaf number and treatment. The main characteristics are discussed very briefly below.

(a) *Total sugar and sucrose.* The following remarks apply equally well to both variables. Leaves 6 and 7 are the only ones in which the content is as high in series P₅K₁ as in P₁K₁, the mean values (leaves 3–10) in series P₅K₁ being 25 per cent. (total sugar) and 29 per cent. (sucrose) below those in P₁K₁. The means of series P₃K₁ are intermediate. Very large interactions occur, and while P₁K₁ and P₃K₁ behave somewhat similarly through the leaf series, the general course in series P₅K₁ is much modified. In series P₁K₁ a fairly uniform decline occurs with leaf number, and the negative correlation is highly significant. A similar fall occurs in series P₃K₁; the data are here more irregular and do not quite attain significance level, but the mean value of P₁K₁ and P₃K₁ falls more regularly with leaf number than does either individually. In series P₅K₁, on the contrary, direct correlation with leaf number is absent, but a parabola with its maximum between leaves 6 and 7 gives a good and highly significant fit. Clearly series P₅K₁ has a lower sugar content than P₁K₁ in early and late leaves, but at the critical period of growth when protein synthesis is minimal, and little carbon is passing into the nitrogen cycle, sugar content rises and becomes approximately normal.

(b) *Reducing sugar.* The general relationships among the reducing sugar data are largely the inverse of those in sucrose. The mean reducing sugar content rises with phosphorus deficiency, the mean of leaves 3–10 being over 20 per cent. higher in series P₃K₁ than in P₁K₁, and over 15 per cent. higher in series P₅K₁ than P₁K₁. In P₅K₁ low values are found in the last two leaves, 9 and 10, and omitting these the value for series P₃K₁ is 23 per cent. in excess of P₁K₁, and for series P₅K₁ 38 per cent. in excess. Again large interactions exist. Thus reducing sugar content in series P₁K₁ rises fairly uniformly and highly significantly with leaf number; that in series P₃K₁ shows a slight trend with leaf number in the direction of a fall; while the relationship in series P₅K₁ is again approximately parabolic. A parabola with its maximum centred about leaf 6 gives a total fit which is just not significant, though the quadratic contribution is itself above significance level.

(c) *Sucrose: reducing sugar ratio.* Since the relationships within the data of one sugar fraction tend to be opposed to those of the other, pronounced variations must exist in the ratio of sucrose to reducing sugar. The treatment ratios are very different, the mean of leaves 3–10 in series P₁K₁ being 65 per cent. greater than that in series P₅K₁, with series P₃K₁ intermediate. The difference between series P₁K₁ and P₅K₁ is highly significant over the whole leaf range with the exception of the last leaf or two, but very considerable interaction is found. In series P₁K₁ the ratio decreases rapidly and highly significantly from leaf to leaf, the last values being less than one half the first. In series P₃K₁ the trend with leaf number is downwards, but is very slight and quite insignificant, while in series P₅K₁ it is again pronounced but reversed, the correlation being highly significant and positive. The results, as regards series P₁K₁ and P₃K₁, agree exactly with those reported by Gregory and Baptiste (1936) (1932 data). Their experiment of 1932 was conducted in a similar manner to the present experiment, and their fully manured and phosphorus deficient treatments were almost identical with the present series P₁K₁ and P₃K₁. Not only is the decrease in the ratio with phosphorus deficiency similar in the two instances, but the type of interaction is identical; the absolute values assumed by the ratio also agree closely. Agreement between the present series P₁K₁ and P₃K₁ and the corresponding series of Gregory and Baptiste is good not only in the sugar ratio, but also in other particulars; this will not be demonstrated here since before comparison can be made it is necessary to recalculate the sugar content data described above in terms of leaf fresh weight.

Finally, there is little evidence in the sugar data of consistent difference due to variation of potassium at the P₃ level.

FACTORS DETERMINING RESPIRATION RATE

The primary object of the present work was to determine as far as possible the respiration changes induced in leaves of the plant by varying the supply of phosphorus in the nutrient over an extremely wide range, and also by

simultaneous variation of both phosphorus and potassium. At the same time, certain nitrogen and carbohydrate fractions were determined on similar material in order to throw as much light as possible on the causes immediately underlying the observed respiratory differences. It would clearly have been desirable to determine the phosphorus and potassium contents of the leaves, but the amount of material needed for these further analyses precluded the attempt. It appears, moreover, that potassium itself exerts no direct effect on respiration rate (Gregory and Richards, 1929; Richards, 1932), so that a knowledge of potassium content is not likely to assist in the elucidation of the immediate question. With phosphorus the state of affairs is different, since there is the possibility of phosphate being concerned directly in the breakdown of the respiratory substrate, while Lyon (1924, 1927*a, b*) has claimed that it is also concerned at a second stage of respiration, i.e. that it is a promoter catalyst in the oxidation phase. It is therefore unfortunate that such data could not be collected, although, as will be seen, the available data appear to be almost sufficient in themselves to account for the observed respiration values. In the following sections the more important inter-relationships between respiration rate and the nitrogen and carbohydrate fractions will be examined and discussed, with the object of determining (1) the extent to which the observed respiration rates may be accounted for by the analytical data, and (2) which of the observed data are most nearly related to respiration. Existing hypotheses will also be examined in the light of the present analysis.

Relationships with amino and protein contents.

In general, nitrogen fractions, protein and amino nitrogen, give higher correlations with respiration than do carbohydrate fractions. This may be partly explained by the fact that the actual leaves used for the determination of respiration rates were themselves included in the samples for nitrogen analyses, while sugars were determined on small parallel samples. In spite of this it appears that in normal leaves taken directly from the plant, before carbohydrate reserves are depleted, respiration rate is more closely related to the nitrogen than to the carbohydrate content. When an attempt is made to distinguish between the relations of protein and amino nitrogen individually to respiration rate, in the high phosphorus series P₁K₁, the same difficulty is encountered as in the data of Gregory and Sen, for among the various leaves protein and amino contents are themselves very highly correlated. All three variables in fact are maximal in the early leaves and minimal in the later. The actual correlation coefficients between the variates, both total and partial, are given in Table XII for all treatments. Coefficients having a significance greater than $P = 0.05$ are indicated by italics.

Clearly in series P₁K₁ it is difficult to disentangle the individual relationships of protein and amino nitrogen to respiration, though there is evidence that the main correlation is with amino nitrogen. In series P₂K₂ this evidence is stronger, while the slight advantage of amino over protein content in series

P₁K₁ is considerably increased by introducing the square of the nitrogen fraction as a second variate, i.e. fitting a parabola to the data instead of a straight line. The extra goodness of fit of the quadratic term from the protein data is negligible, but that from the amino data is highly significant: the multiple correlation R_{R-AA} has the very high value 0.942 (1% point 0.886). There can

TABLE XII

Correlation Coefficients between Respiration Rate, Protein Nitrogen, and Amino Nitrogen Contents

R = Respiration rate, P = Protein nitrogen, A = Amino nitrogen

	RP.	RA.	PA.	5% point.	1% point.	RP.A.	RA.P.	5% point.	1% point.
P ₁ K ₁	+0.767	+0.877	+0.841	0.664	0.798	+0.115	+0.668	0.707	
P ₂ K ₂	+0.478	+0.778	+0.230	0.707	0.834	+0.489	+0.782	0.755	0.875
P ₃ K ₁	+0.766	+0.006	+0.387	0.664	0.798	+0.829	-0.491	0.707	0.834
P ₃ K ₃	+0.595	+0.312	+0.413	0.664		+0.539	+0.089	0.707	
P ₅ K ₁	+0.385	-0.266	-0.461	0.707		+0.306	-0.108	0.755	

be little doubt therefore that amino content is an important factor in determining the respiration rate of leaves from barley at a high level of nutrition, and that the action is similar to that observed when amino acids are fed to plants, as in the experiments of Spoehr and McGee (1923), Genevois (1927), and Schwabe (1932).

It might be assumed from the evidence of series P₁K₁ and P₂K₂ that no very close connexion exists between protein content and respiration rate, most of the apparent effect being derived from the correlation of both with amino nitrogen; but further analysis demonstrates the assumption to be unfounded. Within no other treatment are appreciable effects of amino acids on respiration rate discernible, while between treatments, as phosphorus supply decreases, increasing amino contents are accompanied by decreasing respiration rates. The protein correlation in series P₃K₁, on the contrary, is very close, while that in series P₃K₃ cannot be lightly disregarded. Moreover, the dot diagrams (Figs. 6 and 7), illustrating the relation between the two variables over the whole experiment, demonstrate convincingly that respiration is roughly proportional to protein content over a very wide range of nutrient conditions and throughout the leaf series. A similar diagram for respiration and amino nitrogen brings out clearly the fact that no common proportionality factor exists even for series P₁K₁ and P₂K₂, and in fact within the former treatment the relation has already been shown not to be one of simple proportion. Early leaves at high amino level have a considerably lower ratio of respiration increment to amino content increment than have later leaves at lower levels of amino nitrogen.

In this connexion also, Gregory and Sen (1937) found high correlations with amino nitrogen only in those treatments with low contents; as potassium became more deficient correlations with amino content fell off rapidly until

at very low levels, with high amino contents, all correlation vanished. It is interesting to note that the actual amino contents found in the present series P1K1 and P2K2, where high correlations obtain, fall within the range over which Gregory and Sen obtained a positive effect, while all treatments at lower

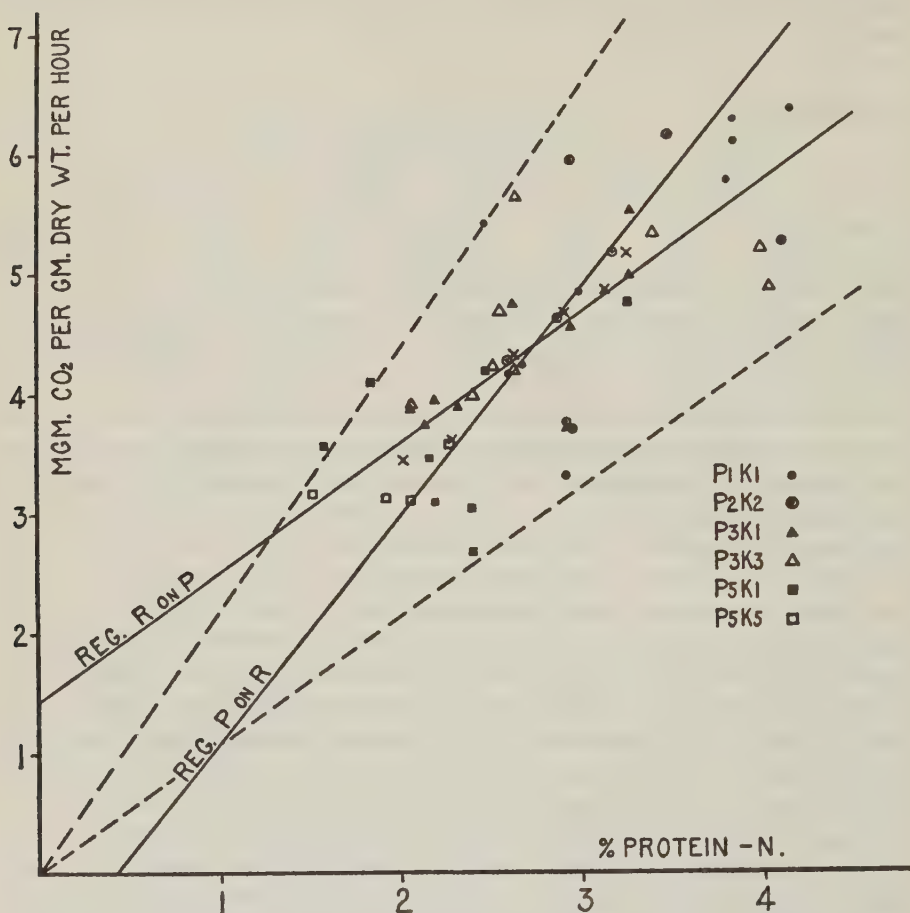


FIG. 6. Correlation diagram between respiration rate and protein nitrogen content: all treatments and leaves. Full lines, regressions of respiration rate on protein nitrogen and of protein nitrogen on respiration rate. Broken lines indicate the limits of error in the ratio of respiration rate to protein nitrogen.

phosphorus levels, giving no correlations with amino nitrogen, have considerably higher amino contents than had even the extreme potassium deficient plants of Gregory and Sen. As has been foreshadowed by the curved regression line found in series P1K1, the explanation is doubtless that the relation between the two variables is of the usual hyperbolic form, and the absence of correlation within treatments with high amino concentrations is not to be interpreted as an absence of effect, but rather that the effect is

everywhere approaching the maximal limit. There is thus little differential effect which alone can lead to high correlation within a series. At low phosphorus levels other factors affecting respiration are in relative minimum; but

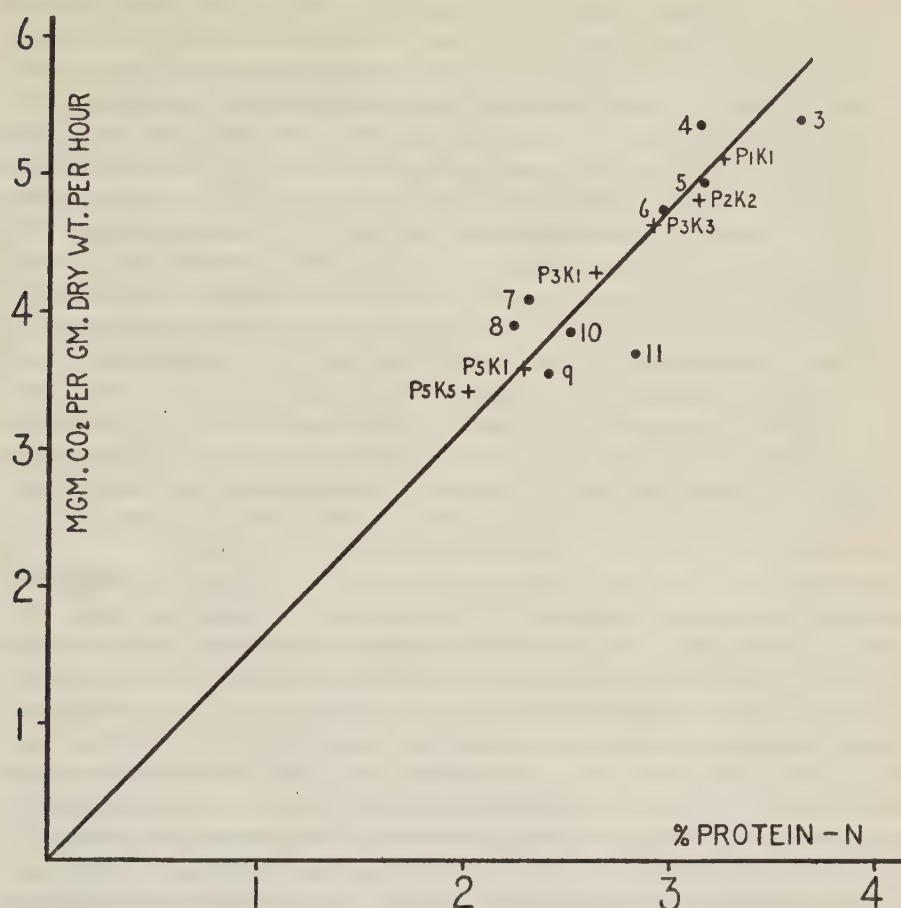


FIG. 7. Correlation diagram between respiration rate and protein nitrogen content: treatment means and leaf means only. Line drawn through general mean and origin.

at high levels, with low soluble nitrogen, these other factors are in excess and respiration rate is then sensitive to changes in amino content.

The relation of protein to respiration rate will be considered further after the carbohydrate relationships have been dealt with; but before leaving the question of the effect of amino content on respiration, reference may be made to the close similarity between the differences in both variables induced by potassium at all levels of phosphorus. At the P_1 level very large increases in both respiration and amino nitrogen are induced by potassium reduction; at the P_3 level comparatively small but real increases in both are obtained by reducing potassium to the same extent as phosphorus; while at the P_5 level

a relatively very great reduction of potassium has no observable effect on either variable. On the other hand, the effects of phosphorus on amino content and respiration rate respectively are opposed, so that while the potassium effects are everywhere characterized by a strong positive correlation between respiration and amino nitrogen, the phosphorus effect is equally well characterized by a negative correlation. Decreasing phosphorus supply leads to progressive decrease in respiration rate and progressive and rapid increase in amino content. A comparison of the present data with those of Gregory and Sen reveals that this inverse relationship with phosphorus holds at all potassium levels, just as the direct relationship with potassium holds at all phosphorus levels. The possibility was suggested previously (Richards, 1932) that the high rates of respiration accompanying reduced potassium supply, all other nutrients being high, may be directly attributable to the increased amino-acid concentration, and indeed a very intimate relation between the two has now been demonstrated by Gregory and Sen (1937). It is natural therefore at first sight to attribute the increased CO_2 production in series P_3K_3 over P_3K_1 to the same cause, and the absence of any potassium effect on respiration at the P_5 level to the absence of an effect on amino nitrogen. While, as has been stated, amino content is likely to affect respiration rate everywhere, the following considerations indicate that the respiratory difference between series P_3K_1 and P_3K_3 is probably not mainly due to this factor.

In series P_3K_3 and P_5K_5 the question is not one of potassium deficiency, as usually understood, but rather of a reduction of excess potassium. For plants of series P_3K_3 have the external appearance ascribed to phosphorus deficiency and by supplying them with extra potassium (P_3K_1) no obvious increase in size or change in external appearance is produced. Potassium cannot therefore be seriously in the minimum, and phosphorus is the nutrient determining size, growth rate, appearance, &c. The increase of potassium from treatment P_5K_5 to P_5K_1 is certainly detrimental in the latter half of the vegetative period, leading to greater retardation of leaf production and shorter individual leaf duration. Under extreme phosphorus deficiency therefore the approximate balancing of potassium with phosphorus is more important than that of potassium with nitrogen. It is probable that similar conditions exist at the P_3 level, although the effect is not so pronounced.

The increased amino content in series P_3K_3 over P_3K_1 cannot therefore properly be attributed to premature breakdown of protein, as it almost certainly must be in a truly potassium-deficient plant (Richards and Templeman, 1936), but is probably little more than an expression of the fact that at the lower potassium level a larger amount of nitrogen has been taken up. For the mean differences between the two series in total protein, and residual crystalloid nitrogen (i.e. non-protein less amino nitrogen) are all in favour of series P_3K_3 , though these differences are not demonstrably significant. The increased respiration rate may be largely explained by the increased level of all nitrogen fractions, i.e. there is a greater quantity of protoplasm respiring;

indeed when measured in terms of protein nitrogen the mean respiration rates of the two series are in close agreement.

Opposed to this hypothesis is the fact that the excess amino nitrogen in series P₃K₃ over that in P₃K₁ is much more uniform from leaf to leaf than that of any other nitrogen fraction, the increase of respiration being similarly uniform; in spite of this, however, evidence of specific effect of amino nitrogen on respiration within either of the treatments is entirely lacking (cf. Table XII), while it will be seen later that respiration in series P₃K₃ may be satisfactorily accounted for by protein and sugar contents. As the comparatively large variations in amino content from leaf to leaf within either series occur without appreciable effect on respiration rate, it is unlikely that the much smaller difference in amino content between the two series can be largely responsible for the observed respiration difference. At the P₃ level amino nitrogen is throughout in large excess and a small difference cannot be expected to have a comparable effect with that of a similar difference at the P₁ level.

For the sake of clarity, in the following analysis and discussion the relation of respiration to protein content will be stressed, and that to amino nitrogen largely ignored, but it should be realized that the relations in series P₁K₁ and P₂K₂ at least are not completely stated without taking into account the effect of amino content.

Relationships with sugars.

An initial survey of the interrelationships between respiration rate, protein content, and the sugar fractions may be made by means of the analysis of covariance. A symmetrical table may be constructed by utilizing eight leaves (leaves 3-10) and five treatments, and the correlations over all 40 points investigated. Similar correlations may then be determined for the leaf totals, treatment totals, and interaction. The results of this analysis are presented in Table XIII.

The multiple correlations demonstrate that the contents of protein and some appropriate sugar fraction account for some two-thirds of the total respiration rate variance over all the forty points, while the respiration rates of the individual leaves in the various treatments, and of the individual treatments throughout the leaf series, are similarly almost entirely accounted for. The high correlation between respiration rate and protein alone is evident everywhere except in the interaction between treatments and leaf number. The total correlation (r_{RP}) between treatments is extraordinarily high; that between leaves is also very high, being nearly complete when the effect of any one of the three sugar fractions is eliminated ($r_{RP,S}$, $r_{RP,S}$, and $r_{RP,T}$). The lack of significance between treatments of the partials $r_{RP,S}$ and $r_{RP,T}$ is clearly due to the high values of r_{PS} and r_{PT} , and indicate that owing to these close correlations it is impossible to separate the protein and carbohydrate effects between treatments. In the interaction there is no evidence of the dependence of respiration rate on protein content, nor indeed on any carbohydrate fraction

TABLE XIII

Analysis of Co-variance, Leaves 3-10, 5 treatments. R and P as before, s=reducing sugar, S=Sucrose, T=Total sugar

(1) Total correlations.									
	RP.	Rs.	RS.	RT.	Ps.	PS.	PT.	5% point.	1% point.
Whole experiment	.	+0.748	+0.009	+0.548	+0.514	+0.288	+0.218	0.312	0.403
Between leaves	.	+0.901	+0.158	+0.548	+0.516	+0.235	+0.184	0.707	0.834
Between treatments	.	+0.973	+0.315	+0.972	+0.981	+0.980	+0.986	0.878	0.959
Interaction	.	+0.104	+0.062	+0.264	+0.254	-0.120	-0.196	0.375	0.479
(2) Partial correlations.									
	RP.s.	Rs.P.	RP.S.	RSP.	RP.T.	RT.P.	Ps.R.	PS.R.	5% point. 1% point.
Whole experiment	.	+0.793	+0.397	+0.524	+0.760	+0.542	-0.507	-0.220	0.316 0.408
Between leaves	.	+0.969	+0.824	+0.798	+0.958	+0.822	-0.828	-0.714	0.875
Between treatments	.	+0.970	+0.076	+0.412	+0.171	+0.565	-0.157	+0.628	0.990
Interaction ¹	.	.	.	.	.	.	-0.511	.	0.487
(3) Multiple correlations.									
	R.Ps.	R.PS.	R.PT.	5% point.	1% point.				
Whole experiment	.	0.793	0.825	0.830	0.47				
Between leaves	.	0.969	0.965	0.969	0.917				
Between treatments	.	0.973	0.978	0.982	0.975 0.995				

¹ All negligible except Ps.R.

This general analysis does not indicate clearly the advantage of one carbohydrate fraction over another in the prediction of respiration rate, though usually total sugar has accounted for slightly more respiration variance than has sucrose or reducing sugar. It is perhaps noteworthy that whereas sucrose content is extremely closely related to respiration rate between treatments (r_{RS}), and fairly closely between leaves and over the whole experiment, the latter relation being highly significant, reducing sugar (r_{Rs}) is nowhere related to respiration unless the effect of protein be eliminated (r_{Rs-P}). This difference is due to the fact that throughout the analysis sucrose tends to be positively correlated with protein (r_{PS}), the relation being exceedingly close between treatments, while reducing sugar tends to vary inversely with protein, the correlation r_{Ps} being significant over the whole experiment and in the interaction. If now the effect of varying respiration rate be eliminated, in all cases except between treatments the correlation r_{Ps-R} is highly significant and negative, while that between protein and sucrose (r_{PS-R}) is nowhere significant, though between leaves it is tolerably high, and everywhere the elimination shifts the coefficient towards the negative side. In the interaction, the negative correlation between protein and reducing sugar is the only relation which is highly significant or even approaches significance level. Additional evidence is therefore adduced to support the contention of Richards and Templeman (1936) and of Gregory and Baptiste (1936) that in phosphorus deficiency the accumulations of amide and reducing sugars are related, and resulting from the fact that protein synthesis has been checked. Since the various correlations between protein and reducing sugar are considerably increased by eliminating the effect of varying respiration rate, an indication is given that reducing sugar rather than sucrose may be the main carbohydrate substrate of respiration.

The interactions of the various fractions between treatments and leaves may be examined by a study of the relationships within the individual nutritional series. The main results of such an analysis are presented in Table XIV. In examining this table it should be remembered that in series P1K1 and P2K2 the main correlations between respiration rate and nitrogen fractions are obtained with amino nitrogen, the protein correlations being lower. For these two series therefore the values of the multiple regressions of respiration on protein and amino contents are also presented. The coefficients between respiration rate and the nitrogen fractions therefore reach a satisfactory level in series P1K1, P2K2, and P3K1, but are low in P3K3, and very low in P5K1.

Hence the first object of the further analysis is to explain the low correlations with nitrogen found under extreme deficiency, and if possible to improve them. It is clear from the table that reducing sugar content is necessary and sufficient for this, and that after eliminating its effect from the respiration-protein correlations in series P3K3 and P5K1 the resulting partial correlations are significant and of approximately the same magnitude as those previously found in series P1K1 and P3K1. Sucrose, on the contrary, is apparently unconnected with respiration rate in extreme deficiency and the respiration-

TABLE XIV
Correlation Coefficients derived from Individual Treatments

(1) Total correlations									
RP.	Rs.	RS.	RT.	Ps.	PS.	PT.	5% point.	1% point.	
P ₁ K ₁	+0.767	+0.831	+0.788	-0.805	+0.377	+0.313	0.664	0.798	
P ₂ K ₂	+0.478	+0.716	+0.699	-0.533	+0.390	+0.340	0.707	0.834	
P ₃ K ₁	+0.766	+0.626	+0.622	+0.226	+0.253	+0.259	0.664	0.798	
P ₃ K ₃	+0.595	+0.276	+0.184	-0.328	-0.084	-0.153	0.664		
P ₅ K ₁	+0.385	+0.441	-0.105	-0.513	-0.676	-0.677	0.707		
(2) Partial correlations.									
RP _s .	Rs.P.	RP.S.	RS.P.	RP.T.	RT.P.	Ps.R.	5% point.	1% point.	
P ₁ K ₁	+0.468	+0.880	+0.911	+0.891	+0.000	-0.577	0.707	0.834	
P ₂ K ₂	+0.642	+0.309	+0.655	+0.357	+0.650	-0.675	0.755		
P ₃ K ₁	+0.760	+0.403	+0.694	+0.800	+0.682	-0.172	0.707	0.834	
P ₃ K ₃	+0.755	+0.616	+0.241	+0.642	+0.347	-0.638	0.707	0.834	
P ₅ K ₁	+0.793	+0.356	+0.105	+0.428	+0.228	-0.824	0.707	0.875	
(3) Multiple correlations.									
	R.P.A.	R.Ps.	R.PS.	R.PT.	5% point.	1% point.			
P ₁ K ₁	0.879	0.783	0.964	0.960	0.795	0.886			
P ₂ K ₂	0.837	0.648	0.748	0.744	0.836				
P ₃ K ₁		0.803	0.887	0.883	0.795	0.886			
P ₃ K ₃		0.777	0.626	0.657	0.795				
P ₅ K ₁		0.837	0.397	0.439	0.836				

protein correlations are not improved by eliminating variation ascribable to it. It may be concluded therefore that under extreme phosphorus deficiency respiration rate is largely determined by reducing sugar content, and in fact in series P5K1 the partial correlation $r_{R_s,P}$ is highly significant and as great as the corresponding partial r_{RP-s} ; the sucrose correlation $r_{RS,P}$ is here completely negligible.

This interpretation of the relationships found is somewhat weakened by the fact that high and significant correlations are found between respiration rate on the one hand and sucrose, and therefore also total sugar, on the other, in the series P1K1. Here the respiration-reducing sugar correlation (r_{R_s}) is significantly 'negative', but this is caused solely by the correlations existing between both and protein content, as is indicated by the negligible value of the partial $r_{R_s,P}$ in this series. The partial r_{RP-s} is also quite insignificant, hence by the method it is impossible to decide whether the high total correlations between respiration rate and both protein and reducing sugar contents are due primarily to a nitrogen or carbohydrate effect. But since the respiration-reducing sugar relation is of an inverse character it is reasonable to suppose that respiration is here determined by the nitrogen fractions and is almost if not quite independent of reducing sugar.

This is not true of the sucrose correlations in the series, for by the elimination of protein the respiration-sucrose coefficient is increased, and similarly elimination of sucrose leads to an increase in the respiration-protein coefficient; taken together the multiple correlation $R_{R,PS}$ reaches the very high value, 0.964. In spite of the high significance it is probable that the respiration-sucrose correlations are here largely fortuitous and mainly due to an unusual chance combination. In no other treatment does the partial correlation $r_{RS,P}$ reach such a very high level, and in precisely those treatments where correlation other than with nitrogenous compounds are needed to account for the observed respiration values, namely series P3K3 and P5K1, sucrose is completely inadequate. On the other hand, as has been shown, variation in reducing sugar content is sufficient to render the results intelligible. Hence, if the observed correlations are accepted at their face value it is necessary to postulate that under complete nutrition sucrose content controls respiration rate and presumably provides the substrate, reducing sugar being without effect, whereas under severe phosphorus deficiency the role of the two carbohydrate fractions is reversed. It is unlikely that this represents the true state of affairs. In fact, if reducing sugar only becomes available for respiration *via* hexose-phosphate, whereas sucrose may provide a substrate capable of immediate attack, as has been suggested (Onslow, 1931; Onslow, Kidd, and West, 1931), it follows that under phosphorus shortage the respiration substrate will be provided almost entirely by sucrose, while at high phosphorus levels reducing sugars may provide much more of the respiratory material, i.e. the tendency of the observed correlations as between series P1K1 and P5K1 would be reversed.

Apart from the difficulty of interpretation of the respiration-sucrose correlation in series P1K1, the evidence of other experiments fails to support the value of the correlation obtained. For example, no such relationship in fully manured plants was found in the previous work of Gregory and Sen (1937); moreover, it is not difficult to adduce contrary evidence from the present data. Thus the eleventh leaf is very small in size and unusually variable; it is normally the last to be produced on the axis, but on occasion may be absent altogether, or alternatively be considerably larger than usual and followed by a minute twelfth leaf. Because of its inherent variability, the low level of most of its important constituents and activities, and the relatively small samples available for the determination of the latter, it is clear that much reliance cannot be placed on statistical results whose magnitude depends largely on this particular leaf. The very high respiration-sucrose correlation in series P1K1 under discussion is in fact determined largely by this leaf. The main values including and omitting leaf 11 are as follows:

	r_{RP}	r_{RS}	$r_{RP.S}$	$r_{RS.P}$
Including Leaf 11	+0.767	+0.831	+0.880	+0.911
Omitting Leaf 11	+0.852	+0.686	+0.892	+0.779

While the fit of the total protein correlation is considerably improved and the coefficient brought up almost to the level of its partial value, the total sucrose correlation is now not significant (5% point 0.707) while its partial only slightly exceeds the 5 per cent. value (0.755). There can be little doubt that these values, omitting leaf 11, give a truer picture of the relationships than when leaf 11 is included. Again, when the part played by amino content is brought to bear on the problem the inconsiderable role of sucrose appears, even including leaf 11 in the calculation. As has been pointed out, amino nitrogen is more highly correlated with respiration than is protein in the series P1K1, and the parabolic regression on amino content alone accounts for 88.7 per cent. of the total variance ($R = 0.942$); better prediction can scarcely be expected in an experiment of this kind. The partial correlation between sucrose and respiration eliminating the linear amino nitrogen term is below significance level, i.e. +0.665 (5% point 0.707), while if the parabolic term be also eliminated there is a further fall to +0.606 (5% point 0.755). Omission of leaf 11 and elimination of the linear amino nitrogen term alone reduces the correlation with sucrose to negligible levels, i.e. +0.338 (5% point 0.755), while the corresponding partial correlation involving amino nitrogen, $r_{RA.S}$, still remains significant (+0.797). It is highly probable therefore that the apparent correlation of sucrose with respiration is mainly traceable to the correlation of both with amino content, and not to a direct effect of sucrose on respiration rate in the series P1K1; the extremely close fit of the regression of respiration rate on protein and sucrose contents is for these reasons regarded as fortuitous.

The experiment as a whole therefore leads to the conclusion that under normal treatment, or treatments involving only slight deficiencies, the respira-

tion rates of young mature leaves taken directly from the barley plant in day-time are dependent almost entirely on some aspect of their nitrogen metabolism, and are nearly independent of their carbohydrate level. Under extreme phosphorus deficiency on the contrary, the content of reducing sugar plays an important part in determining the rates. It may be recalled that previous work (Richards, 1932; Gregory and Sen, 1937) has established the carbohydrate limitation of respiration rate also under extreme potassium deficiency. Here the limitation may be readily ascribed to the low internal sugar concentrations consequent on much reduced assimilation rates; low concentration of substrate is associated with an unusually high potential respiratory activity, leading to direct carbohydrate limitation. *Under phosphorus deficiency the content of reducing sugar which is limiting respiration is as high as that of the high phosphorus plant; with high phosphorus supply the same sugar level not only accompanies a much higher respiration rate, but also exerts no detectable limitation on that rate.* The apparent anomaly may possibly be explained if the hexose-phosphate theory of respiration be accepted, but is more difficult to reconcile with the view which has been put forward by Barker (1936) and Yemm (1935) that the main respiratory substrate consists of active fructose produced by the direct hydrolysis of sucrose.

On the former assumption, the concentration of substrate must depend on the concentrations of both hexose and phosphate, probably in the form of adenylyl pyro-phosphoric acid. With complete nutrition the free phosphate concentration is presumably relatively high and under extreme deficiency greatly reduced, so that the product of hexose and phosphate concentrations must be likewise reduced. If among the various leaves of the deficient plant soon after expansion phosphorus in the hexose-phosphate cycle tends towards a fairly constant low level, the rate of supply of the triose respiratory substrate must be roughly proportional to the hexose concentration, and the demand for substrate be in excess of the supply. At high phosphorus levels on the contrary the supply may be sufficient to meet all normal demands, and reducing sugar apparently bears no relation to respiration rate. Evidence that both sugar and phosphate do exert a direct limitation on respiration rate at very reduced phosphorus levels has been obtained at this Institute (Said, 1934; Sankaran, 1936). Experiments have demonstrated that excised leaves from starved plants show considerably increased respiration rates if instead of dipping into water they are placed with their cut ends in either sugar solution or in neutral phosphate; still higher rates are obtained if sugar and phosphate are presented together. So far as they go, limited experimental data by Said indicate that comparable leaves from highly manured plants under the same conditions are much less responsive, if indeed they respond at all.

The evidence of the present experiment may therefore be construed as supporting the contention of Lyon (1924) that phosphate is normally involved in the respiratory breakdown of sugar in leaves, but little support is found for the view put forward by Onslow (1931) and advocated by Yemm (1935) and

Barker (1936) that half of the sucrose molecule may provide the substrate directly. If this be the correct interpretation it follows that some support is given to the Blackman (1928) view of the identity of the initial stages of aerobic respiration and fermentation, as opposed to the view of distinct origins put forward by Boysen-Jensen (1931) and Lundsgaard (1930). It may be regarded as established that the first stage of fermentation consists in the entry of hexose units into a hexose-phosphate cycle leading to the production of triose. The whole question of the relation between the two types of breakdown is admirably discussed by Turner (1937).

Respiration on a protein basis.

The close relationship between respiration rate and protein nitrogen found throughout the experiment remains to be examined further. The correlation diagram (Fig. 6) shows the general relationship for all observed points, while the corresponding Fig. 7, in which mean values for treatments and leaves are alone plotted, demonstrates in a convincing manner the constancy of the regression over the whole experiment. The straight line in the latter diagram is drawn through the general mean and the origin, and the relation appears to be one of direct proportionality. The only aberrant point is that of leaf 11; as previously mentioned this gives the least reliable data, and moreover it represents a mean of three treatments only, all other leaf points being derived from either five or six treatments. Similarly among the treatment points the most aberrant is P₅K₅, derived from five leaves only instead of from eight or nine as in all others. The larger scatter on the previous diagram (Fig. 6) is by no means entirely due to error, and very considerable improvement may be effected among the points representing series P₃K₃ and P₅K₁ by correcting for variation in reducing sugar content. Fig. 8 gives the result of this correction, together with a corresponding and considerable adjustment for amino N content in series P₂K₂, an adjustment which does not affect the fit of the protein regression. The dotted lines in Figs. 6 and 8 represent the positions of points whose deviation, measured in terms of respiration per unit protein, is twice the standard error. In Fig. 8 not only is the error considerably lessened, but no points in treatments P₅K₁ and P₃K₃ now lie outside the reduced error limits. Of the remaining two series, P₃K₁ has the lowest standard deviation when respiration rate is expressed as CO₂ evolved per unit protein nitrogen per hour, the mean over the whole experiment being in these units 0.165:

	P ₁ K ₁	P ₂ K ₂	P ₃ K ₁	P ₃ K ₃	P ₅ K ₁
σ	0.0265	0.0270	0.0188	0.0284	0.0417.

The value for series P₁K₁ is not appreciably improved by correcting for amino nitrogen, but the error of a single observation is reduced to 0.0217 by omission of leaf 11. In this series protein and amino contents are themselves highly correlated and both are as closely related to respiration rate. Conse-

quently variation due to amino content is completely masked by expressing CO_2 evolution in terms of protein. This holds to a much smaller extent in series P_2K_2 , in which protein and amino contents are only slightly correlated,

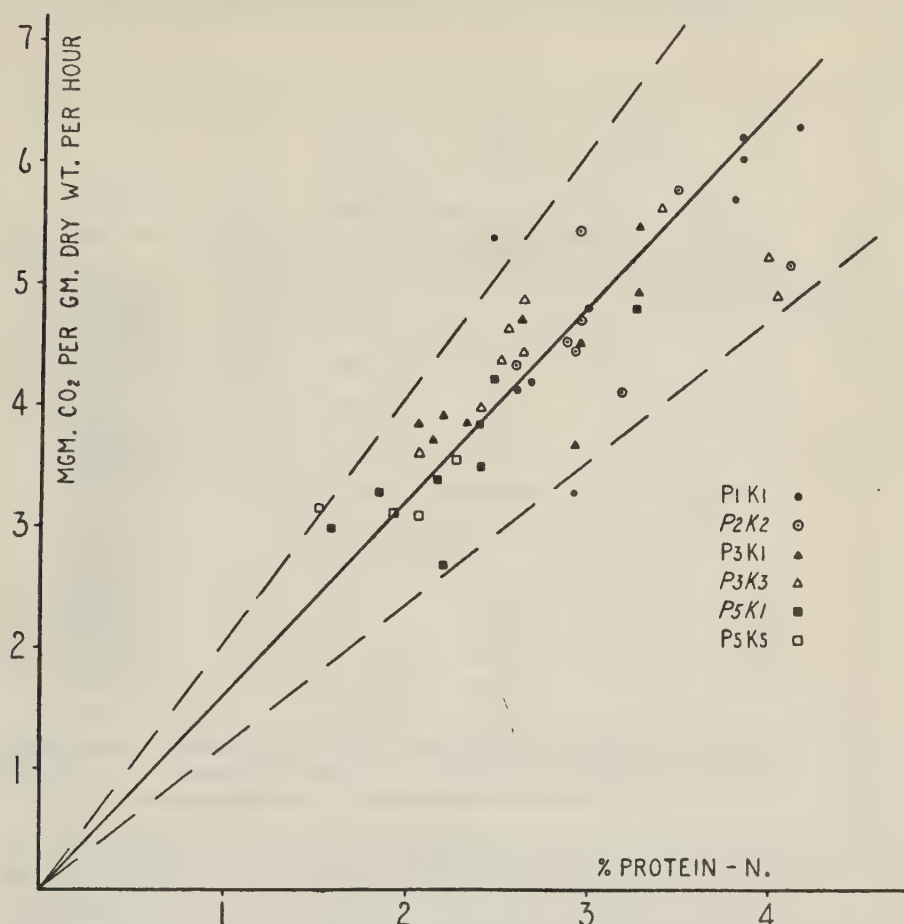


FIG. 8. Correlation diagram between respiration rate and protein nitrogen content: all treatments and leaves. P_1K_1 and P_3K_1 as in Fig. 6. Points in P_2K_2 adjusted for amino-content, and those in P_3K_3 and P_5K_1 for reducing sugar content. Broken lines indicate the limits of error in the ratio of respiration rate to protein nitrogen.

hence respiration per unit protein is correlated with amino content practically to the level of the 5% point, i.e. $r = 0.689$ (5% point 0.707).

In spite of these carbohydrate and amino effects, the mean values for all treatments lie on the same regression line in the dot diagram (cf. Fig. 7). It follows therefore that if throughout the experiment respiration rate be expressed in terms of the amount of protein nitrogen present instead of dry weight the previously established differences ascribable to treatment, and the age drifts with leaf number, almost entirely disappear. This result is of

considerable interest from the point of view of the choice of the best basis on which to place rates of CO_2 evolution for a comparison of the relative respiratory activities of plant organs not nearly identical in composition: and leaves taken from the same plant may show very considerable differences in this

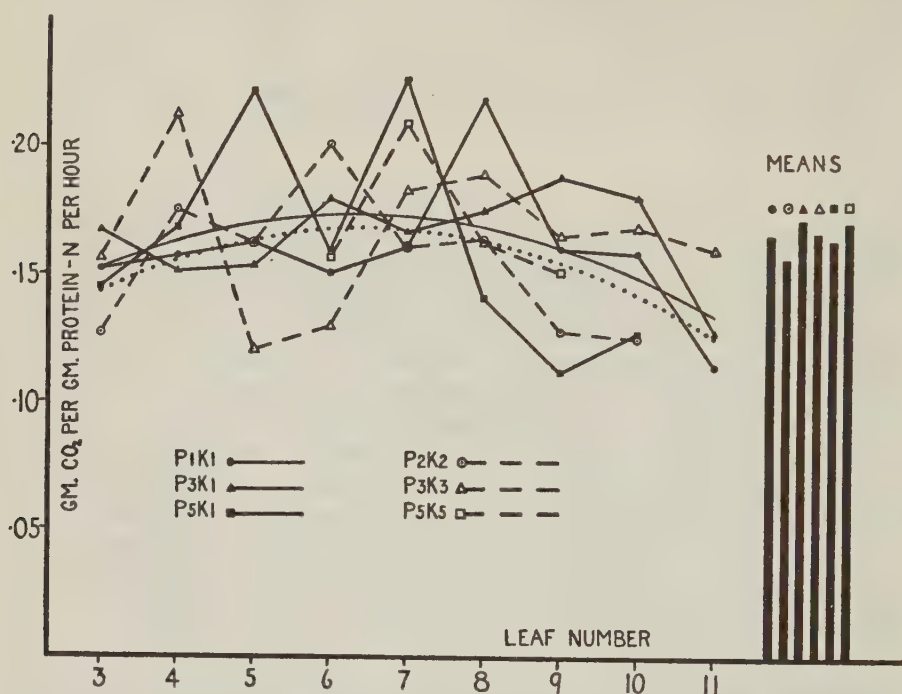


FIG. 9. CO_2 evolved per unit protein nitrogen by successive leaves from the various series, at emergence. Histograms give treatment means. Full curve: regression on leaf number. Broken curve: corresponding regression of reducing sugar content on leaf number.

respect. For instance, in the light of the present result a very large part of the changes found by Kidd, West, and Briggs (1921) in the 'respiratory index' of the various organs of the sunflower throughout life history, must be regarded as simply reflecting a changing content of inert matter in these organs and only in a very limited sense estimating variations in respiratory activity. A basis which automatically removes from the estimation non-living cell constituents must give a measure more easily interpreted than do dry and fresh weights.

In the present instance the values for respiration rate in terms of protein nitrogen are plotted in Fig. 9, and a comparison between this diagram and Fig. 1 reveals vast differences. The variation still remaining may be directly correlated with carbohydrate content, and such an examination completely confirms the relationships between sugar fractions and respiration rates already established by a more circuitous method. Correlation coefficients, both total and partial, are given in Table XV. The first half of the table gives the values of the various correlations when sugar content is expressed in terms

of dry weight. As before, the only values approaching significance for sucrose and total sugar are in the series P₁K₁, but now the simple omission of the doubtful eleventh leaf is sufficient to reduce them well below the significance level and render them comparable with similar correlations in other series.

TABLE XV

Correlation Coefficients between CO₂ per unit Protein (R) and Sugar Content

	P ₁ K ₁ .	P ₁ K ₁ (omitting leaf 11).	P ₂ K ₂ .	P ₃ K ₁ .	P ₃ K ₃ .	P ₅ K ₁ .
R _s . . .	+0.060		+0.526	+0.201	+0.700	+0.839
RS . . .	+0.679	+0.375	+0.473	+0.360	+0.290	+0.502
RT . . .	+0.705	+0.428	+0.493	+0.350	+0.413	+0.568
5% point	0.666	0.707	0.707	0.666	0.666	0.707
1% point						0.834
R _s :S . . .	+0.399		+0.375	-0.071	+0.736	+0.791
RS:s . . .	+0.739	+0.469	+0.278	+0.312	-0.423	-0.233
5% point	0.707	0.755	0.755	0.707	0.707	0.755

Correlation Coefficients between CO₂ per unit Protein (R) and Sugar Concentration

	P ₁ K ₁ .	P ₁ K ₁ (omitting leaf 11).	P ₂ K ₂ .	P ₃ K ₁ .	P ₃ K ₃ .	P ₅ K ₁
R _s . . .	-0.210		-0.275	+0.153	+0.683	+0.830
RS . . .	+0.715	+0.544	+0.026	+0.416	+0.297	+0.459
RT . . .	+0.614		-0.012	+0.386	+0.371	+0.513
5% point	0.666	0.707	0.707	0.666	0.666	0.707
1% point						0.834
R _s :S . . .	-0.571		-0.326	-0.246	+0.755	+0.907
RS:s . . .	+0.810	+0.658	+0.185	+0.452	-0.515	-0.742
5% point	0.707	0.755	0.755	0.707	0.707	0.755
1% point						0.875

At the same time, the reducing sugar effect in low phosphorus treatments is accentuated, and for series P₃K₃ the value is brought above the significance level. In the partial correlations, in which the effect of each sugar is disentangled from that of the other, the reducing sugar correlations in series P₃K₃ and P₅K₁ are unaffected, while the corresponding sucrose correlations are actually negative, demonstrating effectively that sucrose plays no part in respiration under extreme phosphorus deficiency.

It may be objected that real effects of sugar on respiration must depend on their concentration and not on their content in terms of dry matter. In order to meet such objection, so far as is possible, sugar content has been expressed in terms of the amount of water within the leaf, and the above correlations repeated using these data. The results are given in the second half of the same table. It is seen that, far from invalidating the conclusions arrived at, this mode of expression only serves to accentuate the previous findings. The

negative partial correlation r_{RS_s} is here suggestively high in series P_3K_3 , while in series P_5K_1 it practically attains the 5 per cent. level.

It is perhaps of interest to examine the magnitudes of the regression coefficients in series P_3K_3 and P_5K_1 provided by these data. That for the regression of respiration per unit protein on reducing sugar concentration in series P_5K_1 is 0.321, the regression line passing close to the origin, while in series P_3K_3 the corresponding coefficient is much smaller i.e. +0.103. This difference is to be expected if the effect of sugar is more pronounced the greater the degree of deficiency.

The comparatively low value of the regression coefficient in series P_3K_3 provides one possible clue to the small reducing sugar correlations found in series P_3K_1 , at the same phosphorus level as P_3K_3 . Clearly a slightly enlarged error would be sufficient to mask the effect. But error is unlikely to explain the difference, since in series P_3K_1 the observed respiration rates are as well accounted for by protein content as are those in the P_1K_1 series, and the CO_2 evolution per unit protein nitrogen fluctuates only slightly among the various leaves. There appears to be therefore a real difference between the two series at the P_3 level in this respect. The significant difference in respiration rate per unit dry weight, leading to a possibly greater sugar demand in P_3K_3 , cannot account for it: in terms of protein nitrogen, and moreover in terms of reducing sugar, this difference vanishes. A higher phosphate content in series P_3K_1 than P_3K_3 might explain the divergence. The only data bearing at all closely on this point were collected by Shih (1935) at this Institute. He grew barley under almost identical conditions to those of the experiment under discussion, with potassium treatments corresponding exactly with the present levels K_1 and K_3 , and at a phosphorus level which under the present scheme would be designated $P_{2.3}$. Subsequent determinations were made of the total phosphorus content of the whole leaf mass at three periods of growth. Thirty days after germination, $P_{2.3}K_1$ had practically 20 per cent. higher phosphorus content than $P_{2.3}K_3$. This excess gradually disappeared, until by the fifty-second day there was a deficit of 8 per cent. In so far as these results apply to the present problem the indication is that the earlier leaves in series P_3K_1 may have higher phosphorus concentrations than corresponding leaves in P_3K_3 , but that this difference is not likely to be found in later leaves. Here we may recall that the weight of individual leaves, both dry and fresh, is significantly greater in series P_3K_1 than P_3K_3 , maximum differences being found in the earlier leaves, in particular leaves 4 and 5; in later leaves they are very slight. It is probable that a phosphorus effect which tends to disappear in later leaves is represented here, rather than a potassium effect; leaf size is very sensitive to phosphorus, and much less so to potassium.

If this be true, and since in any case the onset of deficiency symptoms is gradual among the successive leaves, it might be expected that series P_3K_1 would show more definite effects of reducing sugar concentration on respiration per unit protein in later leaves than earlier; also that the correlations

would be improved by taking into account a probable increase of effect with leaf number. Considerable correction for any such effect may be made simply by eliminating from the correlations leaf number as a variable. The results are as follows:

Leaves included.	3-II.	4-II.	5-II.	6-II.	7-II.
r_{Rs}	+0.153	+0.200	+0.229	+0.181	+0.258
r_{Rs-L}	+0.227	+0.239	+0.503	+0.841	+0.958

It is seen that whereas the total correlation is not appreciably improved by the successive omissions alone, there is a gradual and pronounced improvement on eliminating general trends with leaf number. The improvement is ascribable to the fact that respiration rate in these units shows a slight tendency to fall in later leaves, while reducing sugar concentration shows a similar tendency to rise. The last two partial correlations reach a high level, but owing to the small number of degrees of freedom available the last is the only one exceeding its 5% point; the previous value, 0.841, falls between the 10% point (0.805) and the 5% (0.878). Somewhat similar improvement, though not so pronounced, may be effected in the same way in the corresponding correlations from series P₃K₃ and P₅K₁, but appreciable reducing sugar relationships appear to be absent from series P₁K₁ and P₂K₂. The coefficient in series P₃K₃ (+0.683) may be improved by omitting the first, or first two, observations, or alternatively by eliminating leaf number; in the latter case the partial correlation derived from all leaves is +0.752, while by omission of the early leaves this may be further raised. The improvement on eliminating leaf number is here due entirely to the increasing concentration of reducing sugar in later leaves; respiration rate has no appreciable correlation with leaf number. In series P₅K₁ again the coefficient of +0.830 rises to +0.886 by eliminating leaf number, while by successive omission of the first three leaves it gradually rises from +0.830 to +0.906. The improvement on eliminating leaf number is in this case ascribable to the fact that respiration rate tends to decline with leaf number, while reducing sugar concentration merely oscillates round a mean value. In all three low phosphorus series then, an effect of reducing sugar may be demonstrated, and again in all three the ratio of respiration per unit protein to reducing sugar concentration tends to fall with leaf number. The difference between series P₃K₁ and P₃K₃ therefore appears to be one of degree rather than of kind.

In Fig. 9 is shown the parabolic regression of respiration per unit protein on leaf number derivable from all leaves in the five main manurial treatments (continuous line), disregarding the manurial differences, i.e. the five observations on any one leaf are treated as independent measures of the same quantity. The curve is apparently real, since the two degrees of freedom representing the total regression have a 'z' value of 0.701 (5% point 0.59; 1% point 0.83); the linear regression has a 'z' value of only 0.131 (5% point 0.70), while the parabolic contribution gives the comparatively high value 0.947 (5% point

0.70; 1% point 0.99). In general, therefore, respiration per unit protein has a slight maximum at leaf 6-7, the latest leaves showing distinctly lower values. Most of this curve is of course accounted for by the reducing sugar relations just discussed, together with the amino content regression in series P₂K₂. Superimposed on the same diagram is the corresponding parabolic regression derived from the reducing sugar contents (dotted line; the vertical scale may here be read directly as % dry weight). Although this regression does not attain significance level the resemblance between the two curves is suggestive. Moreover, the deviations of the respiration data from the former curve and of the reducing sugar data from the latter are also correlated to the level of +0.609 (5% point 0.666).

The respiration data from the whole experiment are therefore very well accounted for by (1) protein content throughout all treatments, (2) amino nitrogen level in the higher phosphorus series, and (3) in the more deficient series, reducing sugar limitation which is apparently characterized by the need of higher sugar concentrations in later leaves than in earlier ones to produce the same respiration rate in terms of protein, and may readily be interpreted as a phosphorus effect. The whole of the reducing sugar data are consistent with the hexose-phosphate theory of respiration, but are not easily brought into line with theories which regard sucrose, or its immediate hydrolytic products, as providing the respiratory substrate without the intervention of phosphate.

In the experiments of Gregory and Sen (1937), respiration rate within the two potassium deficient series was apparently related to sucrose content rather than to reducing sugar. At the same time by treating together the three series at different potassium levels considerably better prediction of respiration may be obtained from the reducing sugar concentration data than from the sucrose concentrations, and moreover the fit so obtained is highly significant and as good as may be expected from the general errors of the experiment. These particular data therefore in no way invalidate the hypothesis that the respiratory substrate is related to reducing sugar and not to sucrose.

The most surprising result which emerges from this experiment is the constancy of respiration rate measured in terms of protein between treatments, and also between leaves when slight adjustments are made for reducing sugar concentration. The rate at 25° C. within a few hours of excision from the plant is approximately 0.165 gm. CO₂ per gm. protein nitrogen per hour, and is maintained both under acute phosphorus starvation, in which respiration rate is known to be 'limited' by both phosphate and sugar concentration, and under high phosphorus supply, in which the rate is not appreciably controlled by these factors. Over the whole treatment range also, amino nitrogen content presumably has a large effect. It would seem therefore that the simple concept of protein nitrogen being only a measure of the mass of protoplasm respiring, or of the enzyme systems concerned, is insufficient to account for the data, and that there must be in fact a reciprocal relation between protein and respiration rate, each being dependent in some measure

on the other. It is true of course that other things being equal the rate of CO_2 evolution will depend simply on the amount of material respiring, and protein may be taken as a rough measure of this. But in the experiment under discussion, both among the extremely different treatments and between successive leaves the other things are very far indeed from equality, and yet the same simple quantitative relation between CO_2 and protein nitrogen holds throughout. It appears highly improbable that under such varied conditions reduction in respiration per unit protein from other causes should always by chance be exactly balanced by increase due to increased amino acid content. But if protein content is itself dependent on respiration the reciprocal relation might well lead to the observed results and moreover help towards an understanding of the cause of the low protein levels and large accumulations of amino acids and asparagine associated with deficiency of phosphorus.

Recently, Gregory and Sen have again drawn attention to this undoubtedly important question, and it is of interest to compare their data with the present result. They worked in the previous year with successive leaves from barley grown under identical conditions to those here described, but with different nutrient treatments. One treatment (N_1K_1) corresponded with the present P_1K_1 , but the variable elements were nitrogen and potassium; their other treatments were N_3K_1 , N_5K_1 , N_1K_3 , and N_1K_5 , the numerals having the same meaning as in this paper. Under nitrogen shortage respiration rate in terms of dry weight was greatly decreased, while under potassium deficiency it was increased. The mean rates of CO_2 evolution per unit protein nitrogen were as follows:¹

Decreasing nitrogen.			Decreasing potassium.	
N_5K_1	N_3K_1	N_1K_1	N_1K_3	N_1K_5
0.357	0.284	0.181	0.236	0.225

It will be seen that the fully manured plants give a *minimum* value which is not far from that found in the present experiment, viz. 0.165. Decreasing nitrogen is now accompanied by rapidly increasing rates until in series N_5K_1 the mean rate is doubled, while in fact in the later leaves of this series the rate is considerably more than doubled. A decrease in potassium also leads to a pronounced rise in the rate, but owing to carbohydrate limitation this is not continued from level K_3 to K_5 . The nitrogen-potassium series is therefore characterized by minimal respiration rates relative to protein at the balanced combination. From the known fact that under phosphorus deficiency respiration rate is very sensitive to supply of both neutral phosphate and sugar, it might be expected that CO_2 evolution per unit protein would here be reduced below that of the fully manured plant. Yet in point of fact protein is always reduced by precisely the same amount as is respiration. Over very wide ranges

¹ The figures given differ slightly from those presented by Gregory and Sen. For comparison with the present data, those given here are derived by determining the rate per unit protein for each leaf and taking treatment means.

of nitrogen, phosphorus, and potassium nutrition therefore the minimal rate of respiration is found in the balanced fully manured plant. This fact may have an important significance, and to maintain a given protein level in the leaf a certain minimal rate of CO_2 evolution may be necessary. Respiration may rise above this level, as under nitrogen or potassium deficiency, but never fall below it; when other causes, e.g. phosphate concentration, operate to reduce respiration, the existing protein cannot be maintained and hydrolysis sets in until protein is again proportional to CO_2 evolution. If this hypothesis should prove to be correct, then the balanced nutrient may be regarded as producing the most efficient plant; nutritive unbalance may lead either to a large amount of 'waste' respiration, or to reduced CO_2 output accompanied by reduced protein synthesis.

SUMMARY

1. A sand culture experiment is described in which barley was grown at three levels of phosphorus nutrition: (1) P_1K_1 the control; (2) P_3K_1 at one-ninth the level of P_1K_1 ; and (3) P_5K_1 at one-eighty-first. In three other series phosphorus and potassium were reduced proportionally: (4) P_2K_2 in which both nutrients were at one-third their levels in P_1K_1 ; (5) P_3K_3 at one-ninth; and (6) P_5K_5 at one-eighty-first.

2. As each leaf on the main axis, with the exception of the first two, reached full expansion, estimations were made of respiration rate (CO_2 evolution), together with certain nitrogen and sugar fractions. Respiration rates were determined in each of two successive periods of three hours shortly after excision; they were re-determined at a later leaf stage. Nitrogen fractions estimated were total, crystalloid, and amino nitrogen, the difference between the first two giving protein nitrogen. Sugars, both total and free reducing, were determined on small parallel samples by the method of van der Plank (1936), the difference giving sucrose.

3. Very considerable treatment differences were obtained in growth rate, final yield, and weight of corresponding leaves. The differences were almost entirely due to phosphorus, and nearly independent of variation in potassium supply at any one phosphorus level.

4. Water content differences were comparatively slight, though real. The relation of phosphorus supply to leaf water content is complex. Proportional reduction simultaneously in both phosphorus and potassium supply leads to no appreciable alteration in water content.

5. Changes in respiration rate undergone during the first few hours in the dark are briefly described, and the results of Gregory and Sen (1937) confirmed.

6. Respiration rate was greatly reduced by phosphorus deficiency, the change with supply being greatest at comparatively high levels. When phosphorus and potassium were simultaneously proportionally reduced, the fall in respiration was at first more gradual than when phosphorus alone was altered, but at lower levels the resultant change was greater. Hence reduction

in potassium at the P₃ level gave a small but real increase in respiration rate, while at the P₅ level no change with potassium was detectable. These results were repeated at the later leaf stage investigated. The correlation surface of the interaction between phosphorus and potassium supply in respiration rate is shown diagrammatically in Fig. 3, and from this it is deduced that all combinations of these nutrients which produce plants having external symptoms associated with phosphorus deficiency also lead to sub-normal respiration rates, while those developing external symptoms of potassium deficiency develop super-normal rates. Respiration differences among the successive leaves are also described.

7. Progressive phosphorus deficiency led to progressive reduction in protein content and progressive and considerable increase in amino nitrogen; total nitrogen was in general minimal at the P₃ level. Potassium reduction at the P₃ level gave a real increase of amino nitrogen, and a similar but insignificant increase of total and protein nitrogen.

8. Total sugars and sucrose were in general reduced by phosphorus deficiency, while reducing sugar was increased, but the relations among the successive leaves were very different at the various phosphorus levels. No consistent differences were found ascribable to potassium. Large treatment differences in the ratio of sucrose to reducing sugar are found, and fully confirm those given by Gregory and Baptiste (1936).

9. The question of the dependence of respiration rate on the levels of the various fractions analysed is fully discussed, with statistical evidence. Respiration was generally closely related to nitrogenous substances, protein and amino nitrogen giving high correlations in series P₁K₁, P₂K₂, and P₃K₁. In series P₃K₃ and P₅K₁ reducing sugar level was also important, this and protein content being the main determinants of the rate. The dependence of respiration on reducing sugar at low phosphorus levels is interpreted as evidence that the substrate is produced from hexose, and that phosphate is necessary for its breakdown.

10. A remarkably close and simple relationship over the whole experiment is described between respiration rate and protein content, after slight adjustments have been made for the level of other factors. To explain this a reciprocal relationship between respiration and protein synthesis is postulated, so that a given rate of CO₂ evolution can maintain only a definite quantity of protein. The hypothesis is also advanced that in the normal plant growing under balanced nutrient conditions efficiency of respiration in this sense is maximal.

The author desires to express his thanks to Sir E. J. Russell for granting facilities to carry out the investigation at Rothamsted; to Dr. H. Said for performing the tedious nitrogen estimations; to Professor V. H. Blackman, and particularly to Professor F. G. Gregory, for many valuable suggestions, continual inspiration, and encouragement.

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NOTES

THE CORRELATION BETWEEN EMBRYO TYPE AND ENDOSPERM TYPE.—During critical and comparative studies of the life histories of plants which have been most commonly investigated, a striking correlation between types of embryo and of endosperm has been found. An explanation—in the real sense of the word—for this is not at present put forward, merely a statement of observed facts.

In those plants in which from the beginning the embryo grows very rapidly and is early differentiated, the endosperm developed in the embryo-sac is almost always non-cellular, cell formation taking place only very late. On the other hand, if the growth of the embryo is very slow or if the ripe seed contains only an undifferentiated embryo, then the endosperm is cellular either from the very beginning, or cell formation takes place very early.

Rapid differentiation of the embryo into cotyledons, plumule, and radicle necessitates rapid consumption of the endosperm, so that increase of non-cellular endosperm is the more economical. Cell formation in the endosperm begins when the embryo is nearing complete differentiation. In the case of slow-growing or undifferentiated embryos, where endosperm consumption occurs late, a more permanent type is formed.

Examples taken from published papers are quoted below: chambered embryo-sacs are chiefly found in saprophytic and parasitic plants, and most of these contain an undifferentiated embryo.

In *Peperomia pellucida*¹ even in oldest fruits, undifferentiated embryos are formed, which are completely surrounded by cellular endosperm.

In *Diospyros virginiana*² the embryo is very late in appearing and remains undifferentiated for a very long time. The endosperm is large and is cellular.

In *Magnolia*³ the cotyledons are initiated after the embryo has enlarged considerably, and the endosperm is cellular from the beginning. The embryo is very small.

Thismia americana.⁴ The seed has a very evident endosperm, with cells of relatively large diameter, and an inconspicuous embryo of a few cells.

Hedyosmum nutans.⁵ The embryo is poorly developed and is simply an oval mass of cells. The endosperm is cellular from the outset.

Linaria vulgaris.⁶ The endosperm is cellular from the beginning, and the majority of seeds are without embryos. The embryo is small and has two short unequal cotyledons.

¹ JOHNSON, D. S., 1900: On the Endosperm and Embryo of *Peperomia pellucida*. Bot. Gaz., xxx. 1-11.

² HAGUE, S. M., 1911: A Morphological Study of *Diospyros virginiana*. Bot. Gaz., lli. 34-44.

³ MANEVAL, W. E., 1914: The Development of *Magnolia* and *Liriodendron*, including a Discussion of Primitiveness of the Magnoliaceae. Bot. Gaz., lvii. 1-31.

⁴ PFEIFFER, N. E., 1914: Morphology of *Thismia americana*. Bot. Gaz., lvii. 122-35.

⁵ EDWARDS, J. G., 1920: Flower and Seed of *Hedyosmum nutans*. Bot. Gaz., lxx. 409-25.

⁶ COOK, M. T., 1924: Development of the Seed of *Linaria vulgaris*. Bot. Gaz., lxxvii. 225-7.

Christisonia.¹ The endosperm is cellular from the beginning and the embryo is undifferentiated and eventually oval in shape.

Sarcodes sanguinea.² The endosperm is cellular from the beginning and is scanty. The embryo is very rudimentary.

Striga lutea.³ The endosperm is initiated by cell formation, and the fertilized egg does not divide immediately.

Eriocaulon septangulare.⁴ In the ripe seed no embryonic organs are recognizable and the endosperm is cellular from the 64-nucleate stage onwards. In most of the Aroideae,⁵ also, the differentiation of the embryo is very late, and correlated with this is the development of a cellular endosperm almost from the beginning. Any number of similar instances can be quoted, and in all these the embryo is deficient in growth and the endosperm is cellular.

Plants in which a completely differentiated embryo is formed are very common, and in these it can be found that the endosperm is nuclear in the early stages, cell formation taking place only late. In order not to increase the bulk of this note only three are quoted.

Impatiens sultani.⁶ The endosperm is nuclear in the beginning, but cellular afterwards. The embryo is fully differentiated and by the time the cotyledons and the radicle are differentiated only two layers of endosperm cells are formed at the periphery of the embryo-sac, the remainder being non-cellular.

Crotalaria sagittalis.⁷ In the embryo the cotyledons are very well advanced. The endosperm is non-cellular and forms a delicate lining for the entire embryo-sac, with considerable masses surrounding the embryo and at the opposite end of the sac. The endosperm practically disappears.

The embryo development of *Gynandropsis pentaphylla*⁸ also is of this type, with a fully differentiated embryo, and non-cellular endosperm.

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RUST PUSTULES ON ROOTS OF ANTIRRHINUM.—In the course of work on antirrhinum diseases in Egypt during the last few years, rust (*Puccinia antirrhini* Diet. and Holw.) was never found until it suddenly appeared in 1936, and was very severe and destructive on a wide range of commercial varieties. Both the uredo, and teleuto stages of spores have been found on the foliage. The fungus

¹ WORSDELL, W. C., 1897: On the Development of the Ovule of *Christisonia*, a genus of Orobanchaceae. Journ. Linn. Soc., xxxi. 576-84.

² OLIVER, F. W., 1890: On *Sarcodes sanguinea* Torr. Ann. Bot., iv. 303-26.

³ MITCHELL, M. R., 1915: The Embryo Sac and Embryo of *Striga lutea*. Bot. Gaz., lix. 124-35.

⁴ SMITH, W. R., 1910: The Floral Development and Embryogeny of *Eriocaulon septangulare*. Bot. Gaz., xlix. 281-9.

⁵ CAMPBELL, D. H., 1900: Studies on Araceae. Ann. Bot., xiv. 1-25.

⁶ OTTLEY, A. M., 1918: A Contribution to the Life History of *Impatiens sultani*. Bot. Gaz., lxvi. 289-317.

⁷ COOK, M. T., 1924: Development of the Seed of *Crotalaria sagittalis*. Bot. Gaz., lxxvii. 440-5.

⁸ RAO, V. S., 1936: Studies on Capparidaceae II. The Embryology of *Gynandropsis pentaphylla*. Journ. Ind. Bot. Soc., xv. 335-44.

was found attacking leaves, stems, bracts, calices, and seed pods. When the season was over, and plants growing in pots were pulled up for examination, teleuto sori were found on the root system of some plants. It was observed on the tap and lateral



roots (see Figure). It was ascertained that the parts of the roots which were found attacked by rust were unexposed to the air and actually covered by the soil.

Rust attack on roots does not seem to have been recorded by Plowright, Grove, McAlpine, or Arthur.

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Studies in the Phytoplankton of the River Thames (1928-1932). I

BY

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With three Figures in the Text

I. INTRODUCTION

OUR knowledge of the phytoplankton of British rivers, and particularly of the larger, slower-flowing streams, is still very inadequate. Fritsch (1902 and 1903) in the early part of the century published the results of a brief investigation of the phytoplankton of the Thames, and later (1905) added notes on the summer plankton of the Cam and Trent. In 1924 Butcher reported on the phytoplankton of the Wharfe (cf. also Schroeder, 1930). All these papers were essentially of a preliminary nature. Butcher (1931, 1932) has published two papers contributing materially to our knowledge of the microflora of the rivers Lark and Tees. They deal chiefly with the attached algae of the river-bed, although the relation between the attached algal flora and the phytoplankton is also discussed (1932). Butcher arrives at the conclusion that the algal plankton is largely recruited from the attached algal community. Mention must also be made of Kofoid's detailed investigation of the plankton of the Illinois (1903, 1908), which extended over a number of years.

It is well known (cf. Butcher (1932), Fritsch (1902, 1903), and the continental work there cited), that the amount as well as the nature of the phytoplankton of a stream depends to a considerable extent on the rate of flow. It seemed, therefore, desirable to obtain more complete data on the phytoplankton of the slow-flowing Thames. The plankton of the latter has consequently been studied at different parts of its course over a number of years in order to determine not only the normal periodicity, but also to establish how far this varies in different years and in different parts of the system. The presence of numerous tributaries and stagnant backwaters, moreover, appeared to constitute a possible source of supply for the phytoplankton, and this question has furnished a further subject for inquiry.

Investigations² with these objects in view were begun in 1928 at the suggestion of Professor Fritsch. Material was collected at fortnightly intervals from

¹ From the Department of Botany, Queen Mary College. In this communication the results are presented in a reduced form. The full data, with many additional tables and graphs, are given in a thesis presented to the University of London.

² They were rendered possible by three grants from the Royal Society without which aid these investigations could not have been carried through.

Rotherhithe (in the London dock area), Kew and Shepperton, although during the winter it was often only possible to obtain material once a month. Collections were made at Kew for three years and at Shepperton for four, while those at Rotherhithe were discontinued after the first year. The plankton was collected with the help of a large net (diam. 25 cm.) of bolting silk (mesh 0.1 mm.) towed from the stern of a rowing-boat for approximately the same length of time, and over the same portion of the river at each collection, at a given station. Since, however, the strength of the current varied considerably from time to time, the volume of water passing through the net was not the same for two successive collections.

II. GENERAL CONSIDERATION OF THE CHEMICAL AND METEOROLOGICAL DATA

(a) *Monthly mean air temperature.* (Data from Kew Observatory.)

The annual sequence for each of the years under consideration was, in general, the same, the coldest month being either January or February, with a mean temperature of 38° or 39° F. and the maximum (62°–65° F.) usually being attained in July. The most striking divergence from the normal was exhibited during January and February 1929, when the mean temperature (32° and 35° F. respectively) was well below the minimum for the other years.

(b) *Monthly mean water temperature.* (Metropolitan Water Board and L.C.C. Records.)

The M.W.B. figures were only available for the period 1928–30, while those of the L.C.C. were incomplete. Generally speaking, the curve of the mean monthly water temperatures closely follows that of the mean air temperatures. The water temperatures usually range from 4° to 18° C., but that of February 1929 was below 2° C.

(c) *Total monthly sunshine.* (Data from Kew Observatory.)

In general the minimum number of hours of sunshine (20–40 hours) was recorded during December or January. The maxima show considerable variation from year to year, and the value decreased from 1928 with 290 hours (in July) to 1931 with 170 hours (in June); 1932 again showed an increase (210 hours in June). The total sunshine for 1929 was, however, greater than in 1928, and was the highest for the five years under review. On the other hand 1931 had an exceptionally low record.

(d) *Rainfall for the Thames Valley above Teddington.* (Data from Meteorological Office Records.)

The general monthly rainfall is very erratic, as Fig. 1 illustrates. Pearsall (1923) has shown that the flood rainfall (i.e. monthly rainfall minus monthly loss of water by evaporation) gives a truer indication of the effect upon a river than the data relating to rainfall alone, the effect of which can partly be

ascribed to its high oxygen content. Since evaporation records for the Thames Valley were not available, the Camden Square data have been utilized to construct the graph (Fig. 1) for the flood rainfall above Teddington.

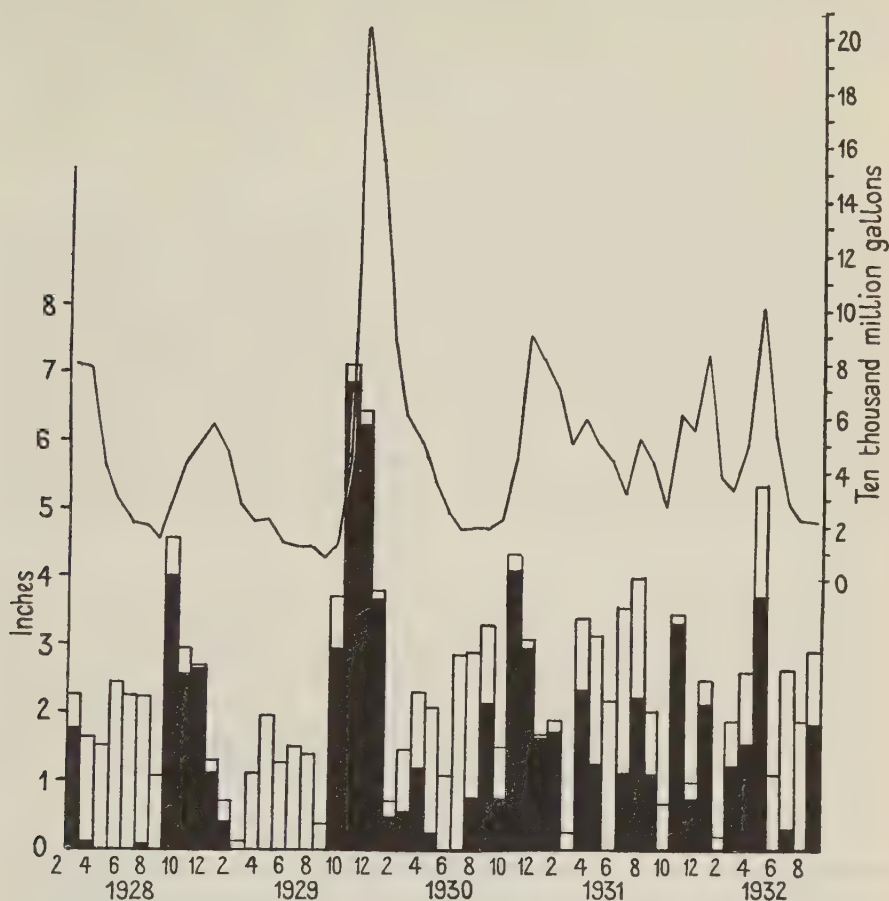


FIG. 1. Rainfall for Thames Valley [■ = flood rainfall]—scale on left, and natural flow of Thames—scale on right.

The significant features of this graph are:

- i. the dry summer of 1929;
- ii. the moderately dry winters of 1928-9 and 1931-2, the former being accompanied by severe frosts;
- iii. the wet summer of 1931;
- iv. the exceptionally high flood rainfall during November and December 1929 (which caused considerable flooding in some reaches of the river), as well as the high records for October 1928, November 1930, and May 1932;
- v. the absence of flood rainfall during June of each year.

(e) *The velocity or natural flow of the river.* (Data from Thames Conservancy Board.)

The velocity of the river is expressed as the total volume of water flowing over Teddington Weir each month, and in general is greatest in winter and least in summer. The most striking figures of the velocity curve (Fig. 1) are:

- i. the exceptionally fast current during December 1929 and January 1930, when the river overflowed its banks and caused extensive flooding of the surrounding country-side;
- ii. the slow current during the summer of 1929;
- iii. the rapid current during May 1932.

There is a general correspondence between the graphs for Velocity and Flood Rainfall except for a marked lag in the velocity curve during the winter months. At these times the velocity continues to increase after the amount of flood rain has commenced to diminish. At other seasons of the year the two graphs correspond much more closely. Although heavy flood rainfall is responsible for an increase in the volume of water flowing over Teddington Weir no close quantitative relation between the two sets of data is apparent. This is no doubt partly due to the fact that, except during periods of exceptionally high rainfall (as in the winter of 1929–30), the volume of water flowing downstream is more or less controlled by the numerous weirs.

(f) *Chemical analyses.*

The Metropolitan Water Board (Houston, 1928–32) undertake regular analyses of the river water at Walton-on-Thames, which is well above the limit of tidal influence, whilst the London County Council make similar analyses from various parts of the tidal region including Kew, which is almost at the limit of tidal influence. The L.C.C. data for Kew were, however, only available for the summer months of the years 1928–31 and are not included in the graphs (Fig. 2).

(i) *Total oxidized nitrogen (nitrates and nitrites)* (Fig. 2). Although there is generally an increase in nitrates and nitrites during or immediately following periods of flood rainfall, at Walton, there is no close correlation between the two sets of data. The maximum content of oxidized nitrogen is usually realized during March, after which the amount steadily decreases until July. After this, there is a further, but by no means steady increase to the March maximum. The exceptionally low content in oxidized nitrogen during the summer of 1929 was undoubtedly due to absence of flood rainfall. At Kew the amount of nitrate and nitrite is usually far in excess of that at Walton. In the summer of 1929, when the amount at Walton was unusually small it was exceptionally large at Kew, which is probably due to sewage pollution.

(ii) *Ammoniacal nitrogen* (Fig. 2). At Walton the maximum amount of ammoniacal nitrogen occurs during the winter months (November to February), and the minimum during summer (May to September). The amount was above the average in the early part of 1929. At Kew the ammoniacal nitrogen

(v) *Turbidity*. At Walton there is a fairly close relation between turbidity and oxygen absorbed from potassium permanganate. The water is most turbid when flood rainfall is greatest; the turbidity being almost wholly due to inorganic and organic debris. The amount of plankton is seldom great enough to impart any marked colour to the water, except, perhaps, for a brownish tinge in spring, when diatoms are particularly abundant. This is in agreement with Kofoid's observations on the Illinois.

(vi) *Hydrogen-ion concentration*. Only the L.C.C. records for Kew were available. Here the pH ranges between 7.25 to 8.5, being highest in spring and lowest in summer or autumn, i.e. the hydrogen-ion concentration is at its lowest at the time of the spring maximum of the phytoplankton. This is no doubt due to the removal of carbon dioxide by the latter, while the presence of large amounts of decaying organic matter in summer is probably responsible for the maximum.

(vii) *Percentage of aeration*. The percentage of aeration at Kew is lowest during the summer months, and appears to be largely determined by rainfall and the amount of organic matter present in the water. It was exceptionally low during the summer of 1929, when there was a low rainfall and a high organic content in this part of the river.

(viii) *Hardness*. At Walton the most marked variation in hardness occurred during 1929. The lower values during the summer months are related to the low flood rainfall. It is, however, difficult to explain the continued decrease in hardness during the winter of 1929 in view of the high flood rainfall.

III. THE PHYTOPLANKTON OF THE MAIN RIVER

(a) *The amount of phytoplankton*.

Since no accurate quantitative estimations were made the amount of phytoplankton present in the diverse collections is only considered from the comparative point of view. Owing to the amount of debris in some of the samples it was wellnigh impossible to count the number of organisms present with any degree of accuracy. The frequencies of the various organisms present in each haul are, therefore, expressed by symbols based on those used by Fritsch, and Rich (1913). The numerical values that have been allotted to these symbols,¹ though arbitrary, approximately correspond with the number of individuals observed when samples of a catch were examined under a $\frac{7}{8}$ in. cover-glass. The numbers thus arrived at have been used in estimating the relative amount of plankton organisms present each month. *Cocconeis placentula* has been ignored in computing these monthly figures, as no free-

	No. of individuals per sample	No. of individuals per sample
Abundant	A = 500	Uncommon U = 25
Plentiful	P = 250	Rare R = 10
Common	C = 100	Scarce S = 5
Frequent	F = 50	Isolated I = 1 to 2

floating frustules of this species were observed in samples of the fresh material (see p. 552).

(i) *Shepperton* (Fig. 3). The maximum abundance of phytoplankton occurs in March and is chiefly due to the preponderance of a few characteristic diatoms, viz.: *Asterionella gracillima*, *Diatoma vulgare*, *Nitzschia linearis*, *Surirella ovata*, *Synedra ulna*, and *Navicula viridula*, while *Melosira varians* and *Synura uvella* (Chrysophyceae) are also quite common. This maximum always occurs very suddenly since the amount of plankton during the preceding months is small. It seems probable that the sudden increase in the amount of plankton, and particularly of diatoms, in March may be related to the considerably increased duration of sunshine in this month. The number of plankton organisms decreases in April, but during May, as a rule, there is again an increase varying considerably in amount from year to year. After this there is a steady decrease until the late summer or autumn, when there is again a slight increase. The number of organisms then decreases to a minimum during the winter months.

The exceptionally high maximum in March 1929 followed a cold, dry January and February; furthermore, the current was unusually slow for this time of the year (Fig. 1). The lowest March maximum occurred in 1932, when the spring was wetter than usual, although the current was slower than during the corresponding month of the previous year.

The amount of phytoplankton present from June to September 1931 was less than in the summers of the other years under consideration. This is perhaps related to the fact that as a result of the higher flood rainfall there was a stronger current during that summer. It is more difficult to find a cause for the general paucity of plankton during the spring of 1932, since in March and April the strength of the current, and other factors (temperature, rainfall, amount of sunshine, &c.) did not differ materially from the corresponding records for the previous year. In May, which was exceptionally wet with an unusually fast current and turbid water, a further diminution in the amount of plankton ensued.

The diatom numbers follow, fairly closely, those of the total phytoplankton. The March phase is due to the sudden increase of species, all of which have been present in small numbers in the preceding months. The number of diatoms always decreases in April followed by an increase in May, which varies in amount from year to year. A much smaller secondary maximum occurs in the late summer or autumn, most marked in October 1931, an unusually dry month.

The marked increase in the amount of plankton in August 1932 was mainly due to green algae, also *Dinobryon divergens*, and may be related to the high mean air temperature (67° F.) during that month. In 1931, with a lower average temperature, the secondary maximum was due almost entirely to increase in diatoms. These differences may also be related to the duration of sunshine.

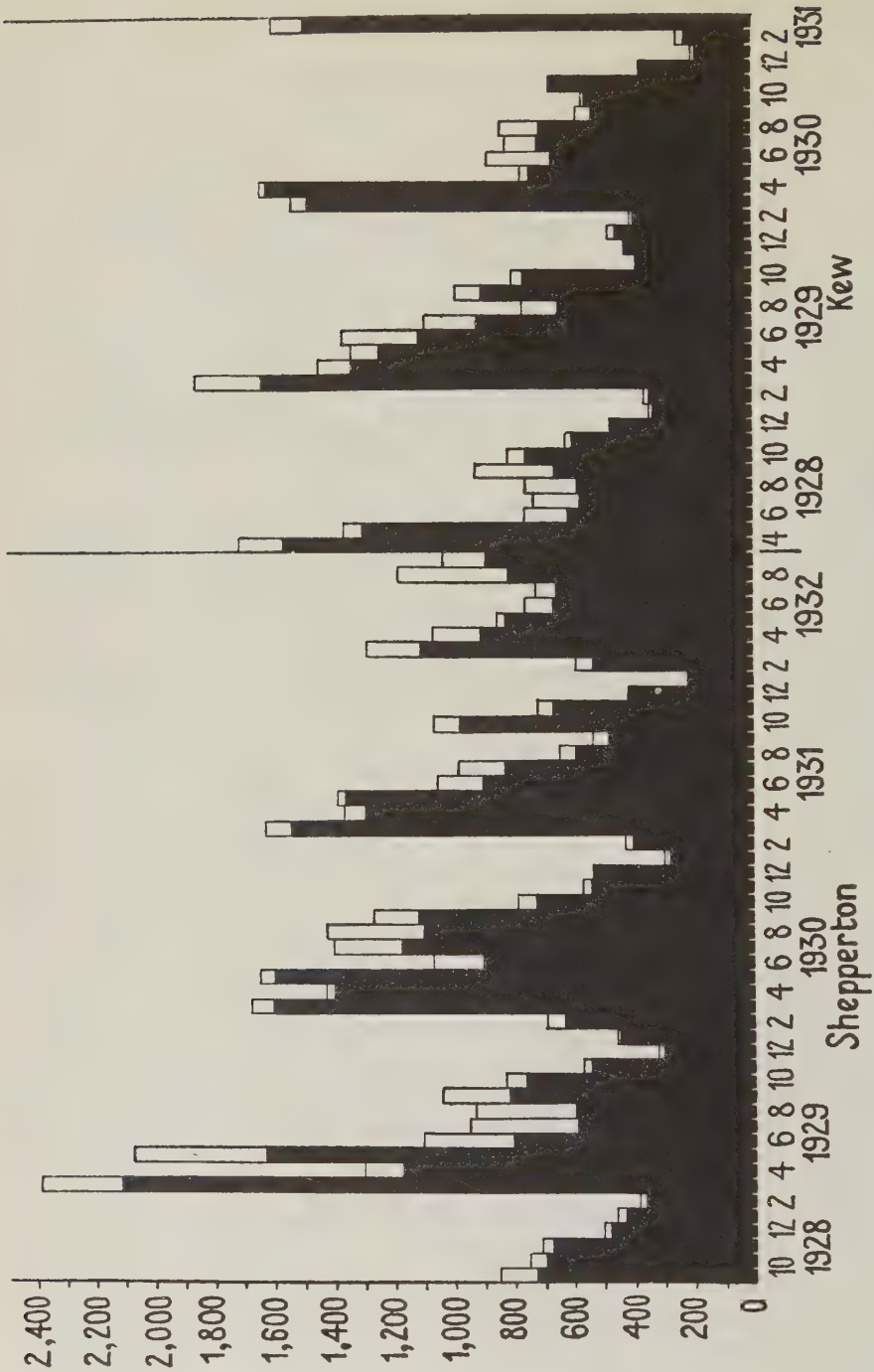


FIG. 3. Total phytoplankton [■ = total diatoms].

(ii) *Kew* (Fig. 3). As at Shepperton the plankton maximum at Kew usually occurs in March, although in 1930 it was deferred until April. There is, however, at Kew no secondary increase of plankton in May. A noteworthy point is the marked difference between the amounts of plankton at Kew and at Shepperton in the summer of 1930. The numbers at Kew in this year remained very low from May onwards. The only significant factor seems to be the low pH value.

The diatom numbers again closely follow those of the total phytoplankton. There is a small secondary maximum in late summer or autumn, which runs parallel with the plankton totals to a more marked degree than at Shepperton.

(b) *Relation between quantity of phytoplankton and environmental factors.*

(i) *Velocity of the current.* There is a definite correlation between the periodicity of the diatoms, and in fact of the whole of the phytoplankton, and a falling water-level, high plankton numbers invariably occurring when the current is relatively slow. The current is usually strongest during December or January, but when the March maximum occurs it has slowed down considerably, though by no means to its minimum rate (Fig. 1). This is in agreement with the conclusions arrived at by Kofoid, and by Pearsall (1930).

The sudden increase of plankton in March does not appear to be due to any considerable extent to detachment of attached species by flood waters, since already in February the velocity of the current has diminished considerably (Fig. 1), whereas the total amount of plankton (and of diatoms) has not yet begun to increase appreciably. In fact at Kew there is little or no increase in the amount either of the total plankton or of diatoms during February. Furthermore, after the high flood rainfall and increased speed of current in May 1932, the total amount of plankton (and of diatoms), nevertheless, diminished steadily until the end of July. Butcher (1932) has already pointed out that there will not in times of flood necessarily be an increase in the proportion of plankton since the volume of water is also increased. The preceding considerations, however, indicate that in a slow-flowing river such as the Thames, despite the probable derivation of the bulk of the plankton from the attached microflora (see Part II), there may, when conditions are suitable, be a considerable increase in the amount of phytoplankton as the result of multiplication of individuals during their passage downstream. Such multiplication was observed in the case of *Synedra ulna*, *Melosira varians*, *Pandorina morum* and *Eudorina elegans*.

(ii) *Oxidized nitrogen and ammonia.* A high content in oxidized nitrogen is associated with high diatom numbers, except when the former occurs during the winter months (Fig. 2). This relation is specially evident in March. There is not, however, a necessary connexion between low diatom numbers and a low nitrate content, although this seemed to obtain during the summer of 1929, when the amount of nitrate was abnormally low (Fig. 2). It thus appears that the nitrate content is seldom low enough to interfere seriously with diatom

development. From March onwards a considerable quantity of nitrates and ammonia must be utilized by the phytoplankton and, owing to lack of rainfall in summer, only diminished supplies will be introduced, so that a low content in oxidized nitrogen and ammonia occurs in summer when there is usually still an appreciable amount of plankton present.

The maximum of ammonia occurs at Shepperton during the winter months (Fig. 2) when there is little plankton. The amount of ammonia seems partly determined by the amount of plankton present, although at Kew it is undoubtedly increased by pollution.

Pearsall (1923), from a consideration of the data available for the Illinois River, concluded that the limiting factor for diatom-development in such waters, which are rich in nitrates, silicates, and organic matter, is much more likely to be some other factor such as dissolved oxygen, the amount of which is high in spring. Judging from the meagre data available for the Thames the percentage of aeration does not shed any light on diatom-periodicity in this case.

(c) *The composition and periodicity of the plankton.*

The mixed marine and freshwater phytoplankton in the river at Rotherhithe is composed in the main of forms which are either dead or in a dying condition. The water here is always extremely turbid and no doubt highly polluted with sewage and other effluents. The large amount of organic matter present is often accompanied by a very low percentage of aeration, while the influx of sea-water at high tides leads to a very high salinity. These factors are evidently not conducive to the well-being of the majority of plankton organisms. The only forms commonly met with in a living condition are (a) the animals *Brachionas pala*, *Eurytemora affinis*, and *Daphnia magna*, the two former often very abundant, and (b) certain diatoms, both marine (*Thalassiosira decipiens*, *Rhaphoneis amphicerus*, and *Nitzschia sigma*) and freshwater (*Cyclotella Kützingeriana*, *C. Meneghiniana*, *Diatoma vulgare*, *Synedra ulna*, *Cocconeis placentula*, *Navicula viridula*, and *N. gracilis*). The marine forms have no doubt been introduced with tidal water, while the others have been carried down from higher reaches. Species like *Cocconeis placentula* and *Navicula viridula* are, however, known to be frequent in such habitats since Butcher (1932) records them from the tidal reaches of the Tees. Certain attached filamentous algae appear to thrive in this region of the river, occurring on the sides of wharves and on piles and steps, which are submerged at high tide but left exposed at low tide. These include *Oedogonium* sp., *Enteromorpha* sp., *Rhizoclonium hieroglyphicum* (with epiphytic *Achnanthes* sp.), *Vaucheria* sp., and *Microcoleus vaginatus*.

The seasonal sequence at Rotherhithe is briefly as follows: From November to February few living forms are encountered; the only common species are *Thalassiosira decipiens* and *Cocconeis placentula*, although the latter is only seen in diatom-preparations (see p. 552). In March *Thalassiosira* be-

comes abundant while *Synedra ulna* is now common. During April *Synedra* increases in amount, becoming the dominant form, while *Thalassiosira* exhibits a marked decrease and *Navicula viridula* becomes prominent. In May *Thalassiosira* and *Navicula* are both common, but *Synedra* diminishes in numbers. During June *Thalassiosira* is still common, while *Nitzschia sigma* springs into equal prominence. In July *Nitzschia* remains common and *Thalassiosira* again increases in amount. Throughout August conditions are much the same except that *Cyclotella Meneghiniana* is now as plentiful as *Nitzschia*. During September all species exhibit a decrease. The most prominent species in October are *Thalassiosira*, *Cocconeis*, and *Cyclotella Kützingeriana*. Although never common *Rhaphoneis amphiceros* is frequent throughout summer and autumn.

The plankton of the non-estuarine part is composed almost entirely of plants. At Kew, near the limit of tidal influence, the plankton generally resembles that of the higher reaches. Apart from seasonal differences (Table and p. 554) its most distinctive features are: (a) the occurrence of a few characteristic marine diatoms, viz. *Coscinodiscus excentricus*, *Nitzschia sigma*, *Thalassiosira decipiens*, *Biddulphia aurita*, and *Rhaphoneis amphiceros*, of which only the first two are usually seen in healthy condition, (b) the presence of *Stephanodiscus astraea*, *Melosira granulata*, and *Ceratium hirundinella*, and (c) the paucity of *Eudorina*, *Pandorina*, and *Closterium* spp.

As in other rivers the dominant forms are diatoms. Species of Chlorophyceae and Chrysophyceae, except at certain times, usually during the summer, are relatively unimportant constituents of the phytoplankton. The paucity of Myxophyceae is worthy of comment. During the autumn, winter, and early spring the character of the plankton is, generally speaking, similar from year to year, but this is not the case during late spring and summer, when there is considerable variation in the relative abundance of the various species.

(i) *The constituent algae*. In the ensuing list the arrangement followed is that of West and Fritsch (1927) except in the case of diatoms, for which Hustedt's (1930, 1930-1) classification has been adopted.

A. CHLOROPHYCEAE (ISOKONTAE)

Chlamydomonas Reinhardtii Dang.

„ spp.

Gonium pectorale Müll.

Pandorina morum (Müll.) Bory.

Eudorina elegans Ehr.

Volvox aureus Ehr.

Pediastrum Boryanum (Turp.) Menegh.

„ *tetras* (Ehr.) Ralfs.

„ *duplex* Meyen.

Micractinium pusillum Fresen.

Lagerheimia genevensis Chod.

Ankistrodesmus falcatus (Corda) Ralfs.

Ankistrodesmus falcatus var. *duplex*
(Kütz.) G. S. West.

Ankistrodesmus falcatus var. *mirabilis*
W. and G. S. West.

Actinastrum Hantzschii (Lagerh.) var.
fluviale Schröd.

Kirchneriella obesa (West) Schmidle.

Dictyosphaerium Ehrenbergianum Naeg
Westella botryoides de Wildem.

Crucigenia rectangularis (Naeg.) Gay.

Tetrastrum staurogeniaeforme (Schröd.)
Chod.

Scenedesmus quadricauda (Turp.) Bréb.

Scenedesmus quadricauda var. *horridus*
Kirchn.

Scenedesmus obliquus (Turp.) Kütz.

„ *bijugata* (Turp.) Lagerh.

- Scenedesmus denticulatus* Lagerh.
Closterium Leibleinii Kütz.
 „ *moniliferum* (Borg.) Ehr.
 „ *Ehrenbergii* Menegh.
 „ *acerosum* (Schrank) Ehr.
Closterium peracerosum Gay var. *elegans*
 G. S. West.
Closterium aciculare Tuffen West var.
subprorum G. S. West.
Closterium siliqua W. and G. S. West.
 „ *gracile* Bréb.
Cosmarium ornatum Ralfs.
 „ *praemorsum* Bréb.
Staurastrum paradoxum Meyen.
Hyalotheca dissiliens (Sm.) Bréb.
- B. XANTHOPHYCEAE (HETEROKONTAE).
Ophiocytium capitatum Wolle.
- C. CHRYSOPHYCEAE.
Chromulina ovalis Klebs.
 „ *microplankton* Pascher.
Mallomonas acaroides Perty.
Synura uvella Ehr.
Dinobryon cylindricum Imhof. var.
palustre Lemm.
Dinobryon divergens Imhof.
 „ *Sertularia* Ehr.
- D. BACILLARIALES (DIATOMALES).
 (1) CENTRALES
Melosira varians Ag.
 „ *granulata* (Ehr.) Ralfs.
 „ *arenaria* Moore.
 „ *sulcata* (Ehr.) Kütz.
Thalassiosira decipiens (Grun.) Joergensen.
Cyclotella Kützingiana Thwaites.
 „ *Meneghiniana* Kütz.
 „ *comta* (Ehr.) Kütz.
Stephanodiscus astraea (Ehr.) Grun.
 „ *Hantzschii* Grun.
Coscinodiscus excentricus Ehr.
 „ *lineatus* Ehr.
Actinoptychus undulatus (Bail.) Ralfs.
 „ *splendens* (Shadb.) Ralfs.
Aulacodiscus argus (Ehr.) A. Schmidt.
Triceratium favus Ehr.
- (2) PENNALES
Diatoma elongatum Agardh.
 „ *vulgare* Bory.
Meridion circulare Agardh.
Fragilaria crotonensis Kitton.
 „ *capucina* Desmazière.
 „ *intermedia* Grun.
 „ *Harrisonii* W. Sm.
 „ *pinnata* Ehr.
Rhaphoneis amphicerus Ehr.
Asterionella gracillima (Hantzsch)
 Heiberg.
- Synedra ulna* (Nitzsch) Ehr.
 „ var. *biceps* Kütz.
 „ *capitata* Ehr.
 „ *acus* Kütz.
 „ var. *radians* (Kütz.)
 Hust.
 „ var. *angustissima* Grun.
 „ *affinis* Kütz.
 „ *pulchella* Kütz.
 „ *parasitica* W. Sm.
Cocconeis placentula Ehr.
Achnanthes microcephala Kütz.
 „ *minutissima* Kütz.
 „ *linearis* W. Sm.
 „ *lanceolata* Bréb.
Rhoicosphenia curvata (Kütz.) Grun.
Frustulia rhomboides (Ehr.) De Toni.
 „ *vulgaris* Thwaites.
Gyrosigma acuminatum (Kütz.) Rabh.
 „ *attenuatum* (Kütz.) Rabh.
 „ *Spenceri* (W. Sm.) Cleve
 var. *nodosa* Grun.
Pleurosigma angulatum (Quekett) W.
 Sm.
Caloneis amphisbaena (Bory.) Cl.
 „ *silicula* (Ehr.) Cl.
 „ *Schumanniana* (Grun.) Cl.
Neidium affine (Ehr.) Cl.
 „ *iridis* (Ehr.) Cl.
Stauroneis anceps Ehr.
 „ *acuta* W. Sm.
 „ *Smithii* Grun.
Navicula cuspidata Kütz.
 „ *mutica* Kütz.
 „ *bacillum* Ehr.
 „ *pupula* Kütz.
 „ *integra* (W. Sm.) Ralfs.
 „ *cryptocephala* Kütz.
 „ var. *intermedia*
 Grun.
 „ *salinarum* Grun.
 „ *rhynchocephala* Kütz.
 „ *viridula* Kütz.
 „ var.?
 „ *hungarica* Grun.
 „ *radiosa* Kütz.
 „ *gracilis* Ehr.
 „ var. *Schizonemoides* V.H.
 „ *peregrina* (Ehr.) Kütz.
 „ *menisculus* Schumann.
 „ *Reinhardtii* Grun.
 „ *placentula* (Ehr.) Grun.
 „ *lanceolata* (Agardh) Kütz.
Pinnularia microstauron (Ehr.) Cl. var.
Brébissonii (Kütz.) Hust.
Pinnularia viridis (Nitzsch.) Ehr.
Amphora ovalis Kütz.
 „ var. *pediculus* Kütz.
Cymbella Ehrenbergii Kütz.

<i>Cymbella prostrata</i> (Berk.) Cl.	<i>Cymatopleura solea</i> (Bréb.) W. Sm.
„ <i>ventricosa</i> Kütz.	„ <i>elliptica</i> (Bréb.) W. Sm.
„ <i>affinis</i> Kütz.	<i>Surirella biseriata</i> Bréb.
„ <i>cistula</i> (Hemprich) Grun.	„ <i>linearis</i> W. Sm.
„ <i>lanceolata</i> (Ehr.) V.H.	„ <i>angustata</i> Kütz.
„ <i>tumida</i> (Bréb.) V.H.	„ <i>robusta</i> Ehr. var. <i>splendida</i> (Ehr.) V.H.
<i>Gomphonema acuminatum</i> Ehr.	„ <i>Capronii</i> Bréb.
„ <i>olivaceum</i> (Lyng.) Kütz.	„ <i>ovata</i> Kütz.
<i>Hantzschia amphioxys</i> (Ehr.) Grun.	„ var. <i>pinnata</i> W. Sm.
<i>Bacillaria paradoxa</i> Gmelin.	„ var. <i>crumena</i> (Bréb.) V.H.
<i>Nitzschia navicularis</i> (Bréb.) Grun.	<i>Campylodiscus noricus</i> Ehr. var. <i>hibernica</i> (Ehr.) Grun.
„ <i>hungarica</i> Grun.	E. CRYPTOPHYCEAE.
„ <i>angustata</i> (W. Sm.) Grun.	<i>Cryptomonas ovata</i> Ehr.
„ <i>dubia</i> W. Sm.	F. DINOPHYCEAE.
„ <i>linearis</i> W. Sm.	<i>Ceratium hirundinella</i> O.F.M.
„ <i>recta</i> Hantzsch.	G. EUGLENINEAE.
„ <i>dissipata</i> (Kütz.) Grun.	<i>Euglena viridis</i> Ehr.
„ <i>acuta</i> Hantzsch.	H. MYXOPHYCEAE (CYANOPHYCEAE).
„ <i>amphibia</i> Grun.	<i>Merismopedia glauca</i> (Ehr.) Naeg.
„ <i>palea</i> Grun.	<i>Microcystis aeruginosa</i> Kütz.
„ <i>communis</i> Rabenh.	
„ <i>sigmoidea</i> (Ehr.) W. Sm.	
„ <i>vermicularis</i> (Kütz.) Grun.	
„ <i>sigma</i> (Kütz.) W. Sm.	
„ <i>parvula</i> Lewis.	
„ <i>acicularis</i> W. Sm.	

A comparison of the lists, giving the commoner members during the period 1928-32 (Table) with the data obtained by Fritsch in 1902-3, reveals that the composition of the plankton has changed considerably during the course of the last thirty years.

The most important differences are as follows:

(1) *Melosira arenaria*,¹ *Cymbella gastroides*, *Fragilaria virescens*, *Tabellaria fenestrata*, and *Grammonema* sp. were all found by Fritsch to be moderately common in the river between Teddington and Kingston, whereas none of these species were found during the course of these investigations.

(2) On the other hand, a number of species now common at Shepperton and Kew, viz.—*Dinobryon divergens*, *Cyclotella Meneghiniana*, *C. Kützingiana*, *Thalassiosira decipiens*, *Diatoma vulgare*, *Fragilaria capucina*, *F. crotonensis*, *Rhoicosphenia curvata*, *Navicula cryptocephala*, *N. viridula*, *Nitzschia linearis*, *N. recta*, *N. sigma*, and *N. vermicularis*—were not recorded by Fritsch from the Thames.

(3) Furthermore, Fritsch's data show that *Campylodiscus noricus*, *Surirella biseriata*, and *S. splendida* (*S. robusta* var. *splendida*) were, during the period 1902-3, common constituents of the Thames plankton, but this was never the case from 1928 to 1932.

(ii) *Seasonal variation at Shepperton* (Table). During December and January *Cocconeis placentula* is the commonest form in diatom preparations. Since, however, no free-floating frustules of this species were ever observed

¹ First recorded as *M. moniliformis*; cf. Fritsch (1905).

during the examination of fresh material, their presence must be due to the occurrence of this species on fragments of macrophytes torn off by the stream and collected by the net. A large number of the diatoms present in midwinter occur only as empty frustules, but a number of living individuals of most species present are always encountered, the most important being *Melosira varians*, *Asterionella gracillima*, *Synedra ulna*, *Navicula* spp., *Gyrosigma attenuatum*, *Nitzschia linearis*, and *N. sigmoidea*; *Asterionella gracillima* was rather common during the winters of 1928-9 and 1929-30. *Synura uvella* is usually present in some numbers in January. During February *Cocconeis placentula* is still frequent in diatom-preparations and *Asterionella gracillima* often rather common, while *Synura* increases in amount.

In March the Spring Phase commences, the commonest diatoms being *Nitzschia linearis* and *Synedra ulna*, while *Navicula viridula*, *Surirella ovata*, and sometimes *Asterionella gracillima* play an important part; *Synura uvella*, *Melosira varians*, and *Diatoma vulgare* are also quite common. All these species have been present previously in small numbers. During April *Nitzschia linearis* is still very common, and in fact often increases in amount, while *Synedra ulna*, and especially *Surirella ovata*, diminish in number; *Asterionella* and *Melosira varians* may exhibit an increase. *Navicula viridula* and *Diatoma vulgare* are still common and *Nitzschia vermicularis* rather common. In some years (viz. 1929 and 1930) *Synura uvella* decreases in numbers. Although the same species are present as in March, their relative abundance varies somewhat from year to year.

In May *Melosira varians* may become one of the dominant species and *Asterionella* is often prominent. *Nitzschia linearis*, *Navicula viridula*, and *Synedra ulna* have as a rule decreased in amount, although the first named is still common; *Diatoma vulgare*, too, remains rather common. A number of green algae (*Eudorina elegans*, *Scenedesmus quadricauda*, and *Closterium Ehrenbergii*) appear during this month, while in 1929 *Dinobryon divergens* was very common.

During June, July, and August a greater diversity of organisms is present, but the summer plankton varies considerably from year to year. In June *Stephanodiscus Hantzschii* is usually common (not 1930 and 1931). *Fragilaria capucina* and *Dinobryon divergens* were exceptionally common in 1929. *Pediastrum Boryanum*, *P. duplex*, *Scenedesmus quadricauda*, and occasionally other green algae (e.g. *Dictyosphaerium Ehrenbergianum*) are rather frequent in the June plankton. During July *Melosira varians* is often very common and in 1930 *Synedra ulna* was codominant with it. In 1932, however, *Stephanodiscus Hantzschii* was the most prominent species. Green algae often play a considerable role during July, *Pediastrum duplex* in 1929, *P. Boryanum* in 1932, and *Closterium moniliferum* in 1931. *Dinobryon divergens* continued to be common in 1929. In August *Melosira varians*, *Synedra ulna*, *Navicula cryptocephala*, *N. viridula*, and sometimes *Dinobryon divergens* are all present in moderate numbers. *Stephanodiscus Hantzschii* was abundant in 1932. The

two species of *Pediastrum* and *Closterium moniliferum* are sometimes well in evidence (especially in 1930 and 1932), while *Pandorina morum* was common at Staines in 1928 and at Shepperton in 1929.

In September *Melosira varians*, *Navicula cryptocephala*, and *Gyrosigma attenuatum* are well represented, while in 1928 and 1929 *Dinobryon divergens* was also common. *Pediastrum duplex*, *P. Boryanum*, *Closterium moniliferum*, and occasionally other green algae (e.g. *Actinastrum Hantzschii* in 1929) are often still present in perceptible numbers. During October *Melosira varians* remains common, while *Synedra ulna*, *Amphora ovalis*, *Gyrosigma attenuatum*, *Navicula cryptocephala*, *N. viridula*, and *N. gracilis* are moderately abundant. *Asterionella gracillima* was very prominent in 1931. As a rule *Cocconeis placentula* springs into prominence in the diatom-preparations in this month. Green algae have usually become rather scarce.

In November *Melosira varians* and *Cocconeis placentula* are usually the two most prominent species, and are accompanied by *Gyrosigma attenuatum*, *Navicula gracilis*, *N. cryptocephala*, and *Nitzschia sigmoidea* in some numbers. *Asterionella* appeared in large quantity in November 1928.

(iii) *Seasonal variation at Kew* (Table I). In December and January, as at Shepperton, *Cocconeis placentula* is the most abundant form in diatom-preparations. *Melosira varians* often persists into December, while *Asterionella gracillima* appears in some numbers at this time of the year. In February *Cocconeis* remains rather common and *Melosira varians* is often well represented, while *Asterionella gracillima* is now usually a prominent form.

With the commencement of the spring phase in March *Synedra ulna*, *Navicula viridula*, *Nitzschia linearis*, and *Surirella ovata* all become very common, while *Asterionella* is now present in considerable numbers; *Nitzschia recta*, *Gomphonema olivaceum*, and *Melosira varians*, as well as two Chrysophyceae (*Synura uvella*, *Chromulina ovalis*), are often rather common. *Chromulina ovalis* and *Gomphonema olivaceum* also occur at Shepperton, but never as plentifully as at Kew, where their prominence is possibly due to pollution; this does not, however, agree with Butcher's (1932) views on the occurrence of *G. olivaceum*. In April *Nitzschia linearis* and *Navicula viridula* are generally codominant. *Asterionella* and *Stephanodiscus Hantzschii* were abundant in 1929, and *Melosira varians* in 1930. *Synedra ulna*, and especially *Surirella ovata*, have decreased in amount. *Diatoma vulgare* is often rather common.

In May *Melosira varians* is usually very common, while *Nitzschia linearis* and *Navicula viridula* have decreased. *Asterionella* is always rather common and was especially prominent in 1929. Other relatively common diatoms are *Synedra ulna*, *Surirella ovata*, and *Diatoma vulgare*. In 1928 *Closterium Ehrenbergii* was very conspicuous.

As at Shepperton the composition of the summer plankton exhibits considerable variation from year to year. In June *Melosira varians*, *Fragilaria capucina*, *Navicula viridula*, and *Stephanodiscus Hantzschii* are all usually

rather common, and in 1929 the last mentioned was abundant. Certain other algae (mainly green), are also on occasion rather common, viz. *Closterium Ehrenbergii* in 1928, *Micractinium pusillum*, *Scenedesmus quadricauda*, and *Dinobryon divergens* in 1929 and *Dictyosphaerium Ehrenbergianum* in 1930. In July *Stephanodiscus Hantzschii* is often one of the most abundant species, while *Pediastrum duplex* may be rather frequent. *Cyclotella Meneghiniana* was common in 1928, *Synedra ulna* and *S. acus* in 1929, while both *Fragilaria crotonensis* and *Ceratium hirundinella* were prominent in 1930. In August *Thalassiosira decipiens*, *Melosira granulata*, and *Cyclotella Meneghiniana* are often present in considerable numbers, while *Pediastrum duplex* is found in some quantity. *Westella botryoides* was common in 1928, *Synedra acus* var. *radians* and *Nitzschia sigma* in 1929, while *Fragilaria crotonensis* and *Ceratium hirundinella* were still prominent in August 1930.

During September *Melosira granulata* remains abundant, while *Thalassiosira* is often less marked. *Cyclotella Meneghiniana*, *Stephanodiscus Hantzschii*, and *Pediastrum duplex* were all common in 1928, *Dinobryon divergens* was prominent in 1929, and *Fragilaria crotonensis* and *Synedra acus* var. *radians* were rather common in 1930. In October *Thalassiosira* usually increases in amount and may become very common, but *Melosira* has diminished. *Asterionella gracillima* is now, as a rule, common. In November the commonest diatoms are usually *Melosira varians* and *Cocconeis placentula*, with *Gyrosigma attenuatum* and *Navicula viridula* in lesser numbers, but in 1929 *Thalassiosira* was the only prominent alga found in the plankton.

The seasonal sequence is thus, generally speaking, the same at Kew and at Shepperton, but there are marked differences in the late summer and autumn, in fact from July to October the dominant species at these two stations are seldom the same. The most striking differences are due to:

- (a) the appearance at Kew of considerable numbers of *Melosira granulata*, *Cyclotella Meneghiniana*, *Fragilaria crotonensis*, and the marine *Thalassiosira*, all of them rare or absent at Shepperton, and
- (b) the greater abundance of *Melosira varians*, *Synedra ulna*, *Navicula cryptocephala*, *N. gracilis*, *N. viridula*, *Gyrosigma attenuatum*, *Amphora ovalis*, as well as of *Closterium moniliferum* and other green algae at Shepperton.

It is possible that the scarcity at Kew of the species enumerated under (b) is due to greater salinity of the water or to pollution, since both factors are always more pronounced during summer and autumn, when flood rain is scarce.

(iv) *Consideration of environmental conditions in relation to phytoplankton during the years 1929 and 1931 at Shepperton.* A consideration of the amount and composition of the plankton in relation to the environmental factors during 1929 and 1931 is of interest on account of the marked differences in climatic conditions, which operated during the spring and summer months of these years.

The environmental factors are summarized in the following table:

Factor.	Period.	1929.	1931.
Total flood rainfall	Jan.-Dec.	17.52 in.	15.60 in.
	Jan.-Sept.	1.53 "	11.51 "
	Mar.-Sept.	—	8.17 "
Total sunshine	Mar.-Sept.	1,386 hrs.	974 hrs.
	June-Sept.	849.5 "	578 "
Average temperature	Mar.-Sept.	55.7° F.	54.2° F.
	June-Sept.	62.0° F.	59.4° F.
Natural flow of river (total)	Jan.-Mar.	130, 180 × 10 ⁶ gal.	202, 261 × 10 ⁶ gal.
	Mar.-Sept.	113, 228 × 10 ⁶ "	331, 892 × 10 ⁶ "
	June-Sept.	42, 581 × 10 ⁶ "	172, 256 × 10 ⁶ "

Monthly averages.

Ammoniacal nitrogen	Mar.-Sept.	0.054 parts per million	0.067 parts per million
	June-Sept.	0.036 " "	0.050 " "
Albuminoid nitrogen	Mar.-Sept.	0.164 " "	0.156 " "
	June-Sept.	0.186 " "	0.158 " "
Oxidized nitrogen	Mar.-Sept.	2.1 " "	2.3 " "
	June-Sept.	1.7 " "	2.1 " "
Oxygen absorbed from KMnO ₄	Mar.-Sept.	1.58 " "	2.49 " "
	June-Sept.	1.74 " "	2.80 " "

The significant differences are:

(a) Practically the whole of the flood rainfall of 1929 fell during the last three months, whereas in 1931 it was more or less evenly distributed. From March to September of 1929 there was no flood rain; on the other hand, in 1931 half the flood rain of the year fell during the corresponding period.

(b) From March to September 1929 there was over 40 per cent. more bright sunshine.

(c) The average temperature from June to September 1929 was 3° F. higher.

(d) The amount of water flowing over Teddington Weir from March to September 1931 was three times as great as during the corresponding period of 1929, while from June to September the amount was four times as great. During the first three months of 1929 the current was unusually slow.

(e) The average amount of ammoniacal nitrogen was from 25 to 40 per cent. greater in 1931, while that of albuminoid nitrogen was 18 per cent. greater during the summer of 1929. During the summer of 1931 there was approximately 25 per cent. more nitrate present.

(f) The amount of oxygen absorbed from acidified potassium permanganate solution was 60 per cent. greater during 1931. This, together with the higher ammonia content, indicates the presence of a greater amount of organic matter, which is to be expected in view of the excessive flood rainfall.

A comparison of the phytoplankton during the two years shows that:

(a) The amount at the spring maximum in 1929 was 50 per cent. more than that in 1931. In 1931 the current at this time was twice as fast as during the corresponding period of 1929.

(b) Algae other than diatoms were poorly represented during 1931. The greater abundance of Chlorophyceae and of Dinobryon from May onwards in

1929 may possibly be related to the prolonged sunshine and (or) the slower current.

(c) The high flood rainfall of August 1931 was accompanied by a paucity of plankton organisms during that and the succeeding month. There was, however, a very marked increase in the amount of diatom-plankton during October, with a complete absence of flood rain. On the other hand, in October 1929 the plankton exhibited a marked decrease in relation to the heavy flood rains which continued until the following January.

(d) There were many differences in detail. Thus *Melosira varians* was abundant throughout the spring and summer of 1931, but was poorly represented in 1929. *Stephanodiscus Hantzschii*, *Dinobryon divergens*, and *Fragilaria capucina* were all prominent during the summer of 1929, but rare or absent in 1931. *Asterionella gracillima*, abundant in the spring of 1929, was prominent only during October of 1931. *Synura uvella*, exceedingly common in March 1929, was only sparsely represented in 1931.

(e) Although there was a general paucity of Chlorophyceae during 1931 *Closterium moniliferum* was well represented in July. The two most common green algae in 1929 were *Pediastrum duplex* and *Pandorina morum*.

(f) The prominence of frustules of *Cocconeis placentula* during the summer of 1931 was undoubtedly due to the scouring action of the stronger current.

From the data available it would appear that sunshine and flood rainfall—with its consequent effect on the velocity of the stream—are responsible for many of the marked differences in the composition and amount of plankton, which are apparent from year to year.

SUMMARY

The phytoplankton of the Thames, like that of other rivers, consists largely of diatoms, and exhibits a well marked seasonal sequence. In January *Asterionella gracillima* is the commonest species, while *Synura uvella* becomes prominent in February. The maximum plankton content is realized in March, the dominant species being *Nitzschia linearis*, *Surirella ovata*, *Synedra ulna*, and *Navicula viridula*, while *Asterionella*, *Synura*, and *Melosira varians* are often well represented. In April *Nitzschia*, *Melosira*, and *Navicula* remain plentiful, but *Synedra*, *Surirella*, and *Synura* decrease in amount, while *Diatoma vulgare* becomes common. In May *Melosira varians* is the dominant species, while *Nitzschia linearis*, and *Navicula viridula* have decreased in number. *Asterionella* may still be prominent. Green algae appear at this time, especially at Shepperton, to become more prominent in June. In the latter month *Stephanodiscus Hantzschii* is often the most plentiful species, and continues to be common in July; at Shepperton *Melosira varians* is sometimes equally abundant.

The most marked difference in the composition of the plankton at Kew and Shepperton is observed in August and the following months. This is probably due to the more marked effect of pollution or to a greater influx of salt water

at Kew, owing to the scarcity of flood water. At Shepperton in August *Melosira varians*, *Navicula cryptocephala*, *N. viridula*, and *Synedra ulna* all occur in moderate numbers, while at Kew the commonest species are *Thalassiosira decipiens*, *Melosira granulata*, and *Cyclotella Meneghiniana*. In September *Melosira varians*, *Navicula cryptocephala*, and *Gyrosigma attenuatum* are common at Shepperton, while at Kew the most prominent species are *M. granulata* and *T. decipiens*. Green algae are now becoming rare. In October *Melosira varians* is plentiful at Shepperton, while at Kew *T. decipiens* is still common and *M. granulata* and *Asterionella* frequent. In November the difference between the plankton at Kew and Shepperton disappears. *M. varians* is still common, with *Gyrosigma attenuatum* well in evidence.

A definite relation between the amount of plankton and a falling water level is evident, the amount being greatest some time after the strength of the current has begun to decrease in the early months of the year. The vernal diatom maximum is always associated with a high percentage of nitrate. A consideration of the plankton in relation to the environmental factors during 1929 and 1931—the former with an unusually dry and hot spring and summer; the latter with a rather wet spring and summer—indicates that sunshine and flood rainfall (with its consequent effect upon the velocity of the river) are responsible for marked differences in the composition and amount of plankton.

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Studies in the Phytoplankton of the River Thames, (1928-1932). II

BY

C. H. RICE

With three Figures in the Text

III. THE PHYTOPLANKTON OF THE MAIN RIVER (*continued from Part I*)

(d) *Diatom periodicity.*

(i) *The periods at which maxima occur.* West (1912), speaking of the English Lakes, states that 'although many of the plankton species of diatoms occur in greatest quantity in spring, some of them attain their maximum in the summer and autumn and several of them have a double maximum one in spring and the other in autumn'. This is equally true of the Thames, as the following list shows:

(1) *Diatoms with spring maximum only.*

<i>Diatoma vulgare</i>	<i>Nitzschia acicularis</i>
<i>Nitzschia linearis</i>	<i>Surirella ovata</i>
„ <i>recta</i>	„ <i>angustata</i>
„ <i>vermicularis</i>	

(2) *Diatoms with summer maximum only.*

<i>Cyclotella Meneghiniana</i> (Kew)	<i>Fragilaria capucina</i>
<i>Stephanodiscus Hantzschii</i>	„ <i>crotonensis</i> (Kew)

(3) *Diatoms with autumn maximum only.*

<i>Melosira granulata</i> (Kew)	<i>Gyrosigma attenuatum</i>
<i>Thalassiosira decipiens</i> (Kew)	<i>Nitzschia sigma</i>
<i>Stauroneis acuta</i>	„ <i>sigmoidea</i>
<i>Navicula cryptocephala</i>	

(4) *Diatoms with a double maximum*, the Spring Maximum being in most cases more pronounced than the other, the time of which is indicated in brackets.

<i>Synedra ulna</i> (late summer or autumn)
<i>Melosira varians</i> (autumn)
<i>Navicula viridula</i> (autumn)

Asterionella gracillima [rather irregular periodicity, usually with maximum in spring and sometimes a second in the autumn (October)]¹

All the above diatoms, with the exception of *Stephanodiscus Hantzschii*, *Stauroneis acuta*, and *Nitzschia acicularis*, are always present.

¹ *Cocconeis placentula* is usually abundant in winter but is not a true planktonic form (Part I, p. 548).

(ii) *The periodicity of certain species.*

1. *Melosira varians* (Fig. 1), which regularly exhibits a double maximum, has a periodicity corresponding closely with that recorded for the Illinois (Kofoid, 1903, 1908). It is least abundant when the strength of the current

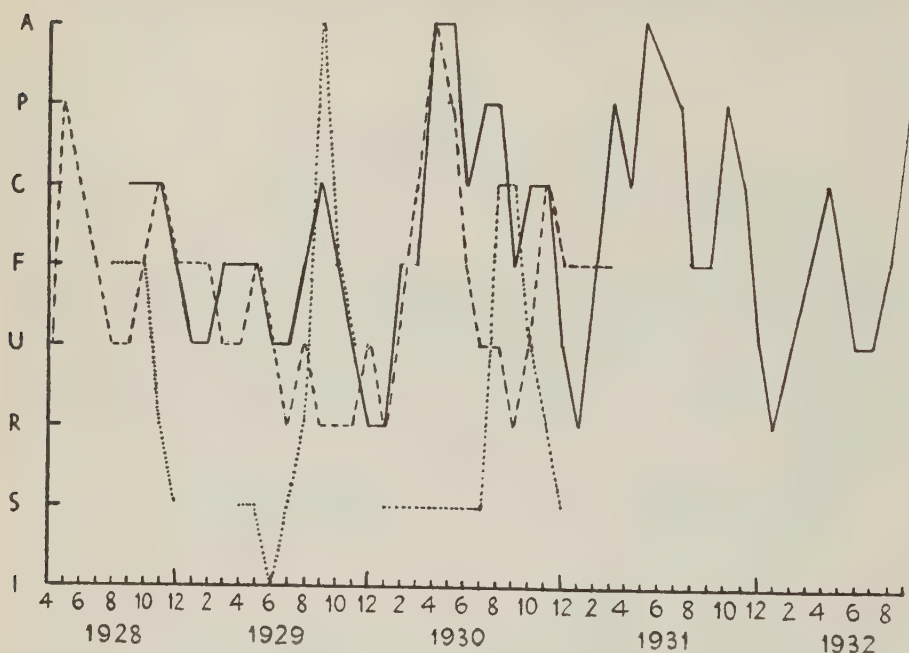


FIG. 1. ——— *Melosira varians* at Shepperton. - - - - *Melosira varians* at Kew.
 *Melosira granulata* at Kew.

is greatest, as is especially well shown at Shepperton during the period 1930–2; in 1929, with a low flood rainfall (from January to September), the abnormally long drought may have been the cause of its scarcity. This species appears to be very hardy, for numbers of healthy colonies were observed in February 1929, when conditions were unusually severe and the surface of the river was frozen over in many places.

2. *Melosira granulata* (Fig. 1), never important at Shepperton, is always prominent during August or September at Kew. Pearsall (1932) found that the occurrence of this species is definitely correlated with water rich in organic matter. Its prominence at Kew may therefore be related to the larger amount of organic matter present.

This species is the most important diatom in the summer plankton of the Illinois at a time when nitrates are at a minimum, and Kofoid assumes that these are utilized by the diatom in question. It was, however, abundant at Kew in September 1929, when the oxidized nitrogen content was abnormally high, and again in August and September 1930, when the nitrate content was

at a maximum. This suggests that the amount of this diatom in the Thames is insufficient appreciably to deplete the nitrate content.

3. *Thalassiosira decipiens*, the only really common marine diatom found at Kew and Rotherhithe, has a maximum at Kew in late summer and autumn, whilst at Rotherhithe it also exhibits a spring maximum. According to Lebour (1930), the maximum occurs in early spring in British seas, but at Kew this spring maximum is doubtless obscured by the greater volume of water flowing down the river at this time of the year. At the time of the Kew maximum the amount of water flowing down the river is at or near its least value and the tidal effect is presumably greatest. The most pronounced maximum was realized at Kew between August and October 1929, after a period of prolonged sunshine and a complete absence of flood rainfall during the summer, so that the tidal effect would no doubt be very marked.

4. *Cyclotella Meneghiniana*, never prominent at Shepperton, was usually rather common at Kew during one of the summer months, being most abundant in 1928. The maxima occur at times of little or no flood rainfall. July 1928 was the sunniest month with the highest mean temperature during the whole period under consideration, and one or both of these factors may have been responsible for the abundance of *Cyclotella*. The greater frequency of this species at Kew may be associated with the greater amount of organic material present.

During August 1928 it was found commonly at Oxford and near Dorchester and also abundantly in the weed-choked North Hinksey stream entering the river at Oxford, in which the floating masses of *Cladophora* were densely covered with a brown scum consisting almost exclusively of this diatom. The frequency of the latter at Oxford can therefore be related to its abundance in the North Hinksey stream. Material was not collected from Shepperton before September 1928, but samples gathered at Staines in August of that year contained only a few individuals of *Cyclotella*. It would appear, therefore, that the abundance of this diatom at Kew was due to contributions from some other source between Staines and Kew. The reasons for the marked reduction in numbers between Oxford and Staines are obscure.

5. *Stephanodiscus Hantzschii* is almost always present, but does not usually attain a maximum until the summer. The highest maxima occurred at Shepperton in 1929 and 1932 and at Kew in 1928 and 1929, all of them years with little flood rainfall during the summer; both 1928 and 1929 had a high sunshine record, but that of 1932 was not as high as that of 1930, when *Stephanodiscus* was poorly represented at Shepperton, although somewhat more plentiful at Kew.

6. *Diatoma vulgare* was abundant only in March 1931. Its periods of frequency correspond with little or no flood rainfall and to a relatively high oxidized nitrogen content. This species was a very common constituent of the brown scum on the slipway at Shepperton Lock boat-house in the spring

of 1929 and on the stones of the river-bed in the spring of 1932. At these times, however, it was not well represented in the plankton.

7. *Fragilaria capucina*, though never abundant, was common at Shepperton in June 1929 and August 1930. It was not so common at Kew, where a maximum was attained in June of the years 1928–30; it is noticeable that the summers of these years had a higher sunshine record than in 1931 and 1932.

8. *Fragilaria crotonensis*, which is almost always present, is never common at Shepperton, but usually exhibits a maximum during July or August at Kew. The reasons for its abundance here in the summer of 1930 are not evident.

9. *Asterionella gracillima* (Fig. 2) has an irregular periodicity. Maxima

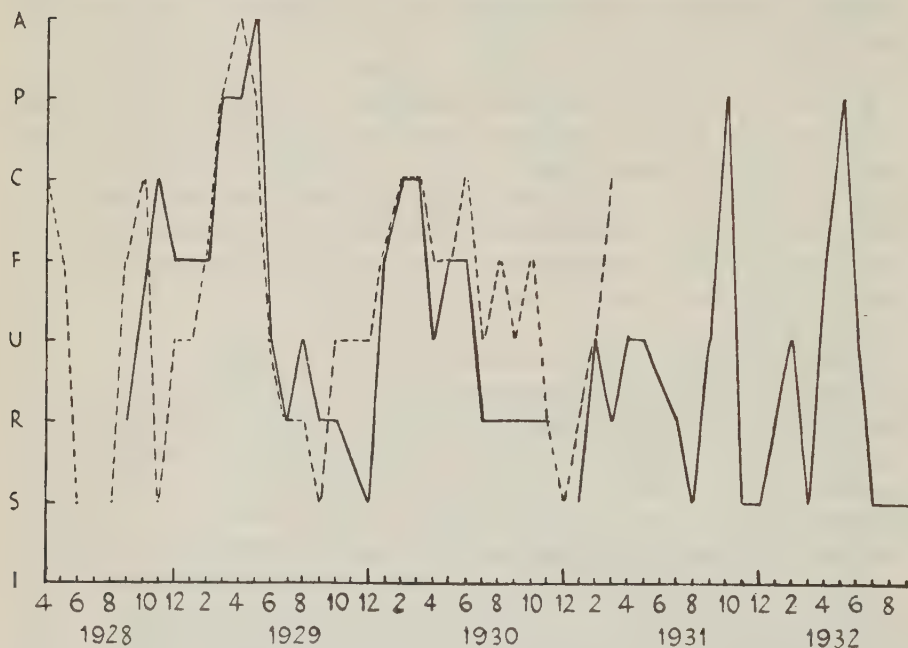


FIG. 2. *Asterionella gracillima*. — Shepperton. - - - Kew.

occur during the colder months (October to May), usually during comparatively dry periods, though this was not the case either in the autumn of 1928 or in May 1932. Scarcity of this species at Shepperton is often associated with a low nitrate content, but since this is not always the case (e.g. not in 1932), some other factor must also be concerned. *Asterionella* generally shows a period of maximum abundance in spring, usually (but not in 1932) when the current is slackening. In 1928 and 1931 it was also common in late autumn (October and November). It is noteworthy that the autumn maximum of 1928 and the spring maximum of 1929 at Shepperton each occurred a month later than at Kew. Furthermore, although *Asterionella* was common at Kew in March 1931, it was poorly represented in the plankton at Shepperton. No explanation of these differences can be advanced.

In the Illinois River, according to Kofoed, *A. gracillima* exhibits a well-marked vernal phase, which is associated with declining floods and a temperature of 60° F. The maxima in the Thames, however, invariably occur at temperatures below 55° F. (in February 1930 below 40° F.). In fact, apart from scarcity of *Asterionella* in summer, its periodicity bears no relation to temperature whatever [cf. Pearsall (1923)].

10. *Synedra ulna* (Fig. 3) is usually at its maximum in March, and there is often a less-marked maximum in June or July, though in 1931 it occurred as

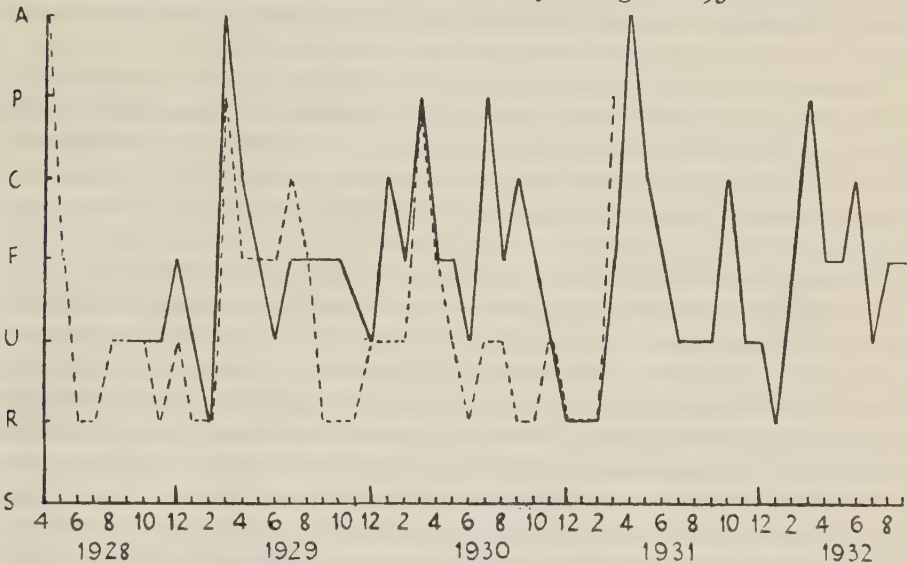


FIG. 3. *Synedra ulna*. — Shepperton. - - - Kew.

late as October. Although the spring maximum corresponds with a high oxidized nitrogen content, that of the summer occurs at a time when the latter is low, so that this factor is obviously never a limiting one. The periods of maximum abundance usually coincide with little or no flood rainfall, but an exception is constituted by April 1931.

Howland (1931) found that the maxima of *S. ulna* occurred when the temperature was low and the salt concentration of the water high. Although this is true of the spring maximum in the Thames, it certainly does not apply to the smaller summer maxima.

At Shepperton in May 1929 and March 1932 and at Kew in September 1928 *Synedra* was found in quantity attached to stones of the river-bed, but only in March 1932 was it simultaneously common in the plankton.

11. *Gyrosigma attenuatum* attains a maximum at Shepperton in September and is much commoner here than at Kew, where the maximum is always later. It is poorly represented in spring. Its greater scarcity at Kew is no doubt due to the special conditions obtaining there (cf. p. 579), but it is impossible to determine the decisive factors. At Shepperton it was really common only in

1928 and 1930, when the amount of organic matter in the water was comparatively small (Part I, Fig. 2), and this may be one of the factors influencing its occurrence.

12. *Navicula viridula* is very prominent in March and April (at Shepperton in 1930 in May), but in 1931 and 1932 there was also a maximum in late summer, less pronounced than the vernal one in 1931, but of equal magnitude to it in 1932. As in the case of other common spring diatoms, the vernal maximum roughly corresponds with the maximum content in oxidized nitrogen. The reasons for the occasional occurrence of a second maximum are not clear.

Large growths of *N. viridula* are especially prominent between March and May, but not at other times of the year, on the sandy bottom in the shallow water on the Middlesex side of the river, about $\frac{1}{4}$ mile below Shepperton Lock. Though equally prominent in the spring plankton at Kew, it was not observed in any quantity on the mud or stones exposed at low tide in this part of the river.

Butcher (1932) records this species as a very prominent member both of the free-floating and of the attached microflora of the Tees and suggests that the river-bed is its normal breeding-place. In the Thames, however, the source of supply does not appear to be restricted to the river-bed (see pp. 572 and 575).

13. *Navicula cryptocephala* is at its maximum in late summer or autumn and at a minimum in spring. Never really common at Kew, it was moderately plentiful in September and October 1928 and August 1930. Its scarcity at Kew in the autumn of 1929 coincides with a low percentage of dissolved air and an increased salinity, both conditioned by lack of flood rainfall. It was abundant at Chertsey and Weybridge in August 1930 and in the North Hinksey branch of the Isis at Oxford in August 1929. At Shepperton, too, it was common only in 1929 and 1930, which had higher sunshine records than 1931 and 1932. In fact, this species appears to become prominent only after a period of prolonged sunshine.

These conclusions do not agree with those of Howland, who found both a spring and autumn maximum at times of high salt concentration. Butcher reports that *N. cryptocephala* in the lower reaches of the Tees shows a maximum only in August. It would thus seem that there is some factor inhibiting the development of this diatom in rivers in spring; perhaps its scarcity, then, is partly a result of competition.

14. *Nitzschia linearis* is always very common in March or April, while in 1929 there was an unexplained minor maximum in October. As in other cases, the spring maximum corresponds with a maximum content in oxidized nitrogen, while periods of scarcity are usually associated with low nitrate values.

15. *Nitzschia sigma* occurs only in the tidal reaches of the river, where it is found all the year round. Carter (1933) records it from the mud-flats at Canvey Island in the Thames estuary. It is common at Rotherhithe and,

though never common at Kew, it is usually more abundant there during summer or early autumn, which is possibly due to the more marked tidal effect.

16. *Nitzschia acicularis* occurs in the plankton only in spring and early summer with pronounced maxima at Kew in April 1928 and May 1929; at Shepperton it was common only in May 1929. Butcher records *N. acicularis* as an important member of the attached microflora of the Tees and other rivers, with a pronounced maximum in May. If the river-bed is the normal breeding-ground of this species one would expect some relation between the amount of it in the plankton and the strength of the current. At the time of the maxima in the Thames there was an almost complete absence of flood rainfall, although in 1928 the current was much faster and in 1929 considerably slower than in other years.

17. *Nitzschia vermicularis* is frequent only at the time of the spring maximum.

18. *Nitzschia sigmaidea*, although always present, is never common. At Shepperton it is usually most plentiful in late autumn or early winter, but in 1930 and 1931 there was also a spring maximum. This diatom generally becomes commoner after heavy flood rainfall, and its greater abundance in the spring of 1930 and 1931 was no doubt related to the high rainfall during the preceding winters.

19. *Surirella ovata* always shows a maximum in spring, usually in March when there is a high nitrate content; after that it decreases very rapidly in amount. The most marked maximum in March 1929 coincided with a higher sunshine record than usual.

S. ovata is recorded by Butcher as a prominent member of the microflora of the bed of the Tees. This species was always commonly found in the scum on piles, slipways, stones, &c., in spring, although subsequently decreasing in amount, so that its periodicity as an attached form is similar to that in the plankton.

(e) Periodicity of the Chlorophyceae.

All the common species of Chlorophyceae are essentially summer forms, which in part persist until the autumn. The data given below indicate that for many of them prolonged sunshine leads to maximum abundance.

1. *Pandorina morum* occurred commonly only in August of 1928 (at Staines and near Dorchester) and 1929 (at Shepperton, at Oxford, and near Dorchester), the previous month in each case having a high sunshine record (250 hours) and scanty rainfall. On the other hand, it was completely absent in 1931 and rare in 1930 and 1932 with little sunshine in July. When frequent numerous reproducing colonies were always observed.

This is not in agreement with Fritsch and Rich (1913), who concluded that abundant sunshine was detrimental to the development of this species, while Howland found it to be abundant during a period of abnormally high rainfall. Hodgetts (1921 and 1922) concludes that the periodicity of *P. morum* is not

related either to temperature or sunshine but that in ponds competition may determine its frequency. These conflicting conclusions suggest that the determining factor has yet to be discovered.

2. *Eudorina elegans* appeared every spring and persisted through the summer, but was never common, though reproducing colonies were often observed.

3. *Pediastrum duplex* is generally most prominent from June to August after periods of prolonged sunshine, the maximum occurring in July or August. It was abundant in August of 1928 at Abingdon and Oxford and near Dorchester after previous prolonged sunshine, while at Shepperton in 1931, with a low sunshine record, it appeared only in small quantities for a very short time. Its poor representation in 1930 is, however, not accountable on this basis. *P. Boryanum*, although a regular constituent of the summer plankton, is never as common as *P. duplex*. It occurred most abundantly at Shepperton during August of 1932, which was unusually hot.

4. *Scenedesmus quadricauda* is usually present from spring to autumn, although in 1931 and 1932 it was rare and only found from May to July and June to September, respectively. These two years were less sunny than the others. *S. obliquus* was never more than rare.

5. *Closterium moniliferum* is a regular member of the summer plankton at Shepperton, but is rare at Kew. The maximum usually occurs in August, but in 1931 it was realized in July with a second maximum in October.

Periods of maximum abundance of this species correspond with adequate sunshine and a moderate amount of flood rainfall which possibly carries it down from breeding-grounds in the upper reaches of the river.

C. Ehrenbergii is invariably present during spring and summer but was common only in the spring of 1928 at Kew. Butcher records this species as a member of the bottom flora of the Tees, so that the faster current in the spring of 1928 may have detached individuals from the bed of some shallow nearby tributary.

C. acerosum, though usually found during spring and summer, is seldom common.

(f) Periodicity of the Chrysophyceae.

1. *Synura uvella*, a common member of the plankton, is usually absent from June to August. This is in agreement with the known preference of most Chrysophyceae for colder waters; Kofoid too found *S. uvella* to be the most important winter plankton of the Illinois. Its maximum is usually in March, although it is well in evidence during February; it was abundant in March 1929 after the coldest winter of the period under consideration and was also common in the spring of 1932 after a cold February. In autumn there is a much smaller secondary maximum. This species was, however, absent during the winters 1930-1 and 1931-2, so that other factors are evidently also involved.

2. *Dinobryon divergens* was really prominent only during the summer of 1929, with a complete absence of flood rainfall and a high sunshine record. Owing to lack of flood rainfall the oxidized nitrogen content at Shepperton fell to its lowest value during this summer, while the albuminoid nitrogen was higher than in all the other summers except 1931. Abundant representation of this species appears, therefore, to depend on the presence of a certain percentage of organic matter in conjunction with abundant sunshine.

Pearsall (1932) states that *D. divergens* is favoured by a high nitrate/phosphate ratio. His data do not exhibit any relation between the occurrence of this species in lakes and the amount of organic matter present.

(g) *Summary of the factors apparently affecting the periodicity of the more important members of the Thames plankton.*

The suggestions put forward below are tentative and by no means conclusive.

1. *Temperature.*

(a) Organisms favoured by low temperature: *Synura uvella*.

(b) Organisms favoured by a high temperature: *Pediastrum duplex*, *P. Boryanum*, *Cyclotella Meneghiniana*.

(c) Organisms adversely affected by high temperature: (*Asterionella gracillima*?)

2. *Sunshine.*—Organisms favourably affected by sunshine: *Pandorina morum*, *Pediastrum duplex*, *Scenedesmus quadricauda*, *Dinobryon divergens*, *Cyclotella Meneghiniana*, *Fragilaria capucina*, *Navicula cryptocephala*.

3. *Flood rainfall.*

(a) Organisms favoured by low flood rainfall: *Pandorina morum*, *Cyclotella Meneghiniana*, *Stephanodiscus Hantzschii*, *Diatoma vulgare*, *Synedra ulna*.

(b) Organisms adversely affected by low rainfall: *Closterium moniliferum*, *Melosira varians*.

(c) Organisms favoured by high rainfall: *Nitzschia sigmoidea*.

4. *Current.*

(a) Organisms occurring when the current is strong: *Closterium Ehrenbergii*, *Cocconeis placentula*.

(b) Organisms adversely affected by a strong current: *Melosira varians*.

5. *Nitrates.*—Organisms favoured by a high percentage of nitrate: *Melosira granulata*, *Diatoma vulgare*, *Synedra ulna*, *Navicula viridula*, *Nitzschia linearis*, *Surirella ovata*.

6. *Organic matter.*

(a) Organisms favourably affected: *Melosira granulata*, *Dinobryon divergens*.

(b) Organisms adversely affected: *Amphora ovalis*, *Gyrosigma attenuatum* (or low percentage of aeration).

IV. THE PLANKTON OF TRIBUTARY STREAMS AND BACKWATERS

During August of 1929 collections were made over a period of a week from various parts of the river (viz. Kew, Shepperton, Windsor, Bourne End, Reading, Dorchester, Oxford, Lechlade), in order to determine whether there was any appreciable variation in the plankton in the different reaches at this time of the year. Examination of the material (Table I) showed that, although

TABLE I
The Phytoplankton of the Thames, August 1929

	9th	8th	10th	15th	13th	12th	14th
	Shepperton	Windsor	Bourne End	Reading	near Dorchester	Oxford	Lechlade
<i>Pandorina morum</i>	F	U	F	F	C	C	U
<i>Scenedesmus quadricauda</i>	F	R	R	S	R	R	U
<i>Dinobryon divergens</i>	U	U	S	F	U	S	—
<i>Melosira varians</i>	F	C	C	R	C	F	R
<i>Cyclotella Meneghiniana</i>	R	R	U	U	R	U	C
<i>Stephanodiscus Hantzschii</i>	U	S	S	S	R	F	—
<i>Fragilaria capucina</i>	C	C	F	U	C	C	R
<i>Synedra ulna</i>	U	U	U	U	U	F	U
„ <i>acus</i>	U	U	F	U	U	R	R
<i>Navicula cryptocephala</i>	U	U	C	U	U	F	F
„ <i>viridula</i>	F	S	S	S	S	S	R
„ <i>gracilis</i>	U	U	U	S	F	U	F
„ <i>radiosa</i>	S	—	S	P	S	S	S
<i>Amphora ovalis</i>	U	U	F	R	F	S	F
<i>Nitzschia linearis</i>	U	F	U	R	U	R	F
„ <i>recta</i>	S	S	S	R	R	R	F

the general character of the plankton was approximately the same from Shepperton to Lechlade,¹ yet certain species common to one reach were rare or absent in others. The following instances may be given.

The most striking example of such local differences was found at Reading above the entry of the Kennet; here *Navicula radiosa* occurred in large quantity although it was very rare in other stretches. Similarly at Lechlade *Nitzschia recta* and *Cyclotella Meneghiniana* (cf. also p. 561) and at Shepperton *Navicula viridula* and *Scenedesmus quadricauda* were considerably commoner than elsewhere.² Other species that showed considerable variation in relative abundance at different points at which collections were made were *Pandorina morum*, *Dinobryon divergens*, *Melosira varians*, *Stephanodiscus Hantzschii*, *Fragilaria capucina*, *Navicula cryptocephala*, *Nitzschia linearis*, and *Amphora ovalis*.

¹ The plankton at Kew has been omitted from this comparison owing to the obvious tidal effect obtaining there.

² It is also worthy of note that floating masses of *Oscillatoria limosa* were plentiful at Bourne End, Reading, and Oxford, while *Enteromorpha intestinalis* was common at Lechlade.

The commonest species at each point were as follows:

Shepperton: *Pandorina morum*, *Scenedesmus quadricauda*, *Melosira varians*, *Fragilaria capucina*, and *Navicula viridula*.

Windsor: *Melosira varians*, *Fragilaria capucina*, and *Nitzschia linearis*.

Bourne End: *Pandorina morum*, *Melosira varians*, *Fragilaria capucina*, *Synedra acus*, *Navicula cryptocephala*, and *Amphora ovalis*.

Reading: *Pandorina morum*, *Dinobryon divergens*, and *Navicula radiosa*.

Near Dorchester: *Pandorina morum*, *Melosira varians*, *Fragilaria capucina*, *Navicula gracilis*, *Amphora ovalis*, and *Cymbella affinis*.

Oxford: *Pandorina morum*, *Melosira varians*, *Stephanodiscus Hantzschii*, *Fragilaria capucina*, *Synedra ulna*, *Navicula cryptocephala*, and *Nitzschia communis*.

Lechlade: *Cyclotella Meneghiniana*, *Navicula cryptocephala*, *N. gracilis*, *Amphora ovalis*, *Nitzschia linearis*, and *N. recta*.

It is quite clear, therefore, that, in the summer months at least, the composition of the plankton is by no means uniform, but shows local variations that can only be due either to local factors or to a local supply of material from tributary streams or backwaters. In August 1930, consequently, an endeavour was made to establish more fully the sources from which the summer plankton of the main river is recruited. With this object in view samples were collected from diverse backwaters (Eton, Chertsey, Weybridge, Shepperton, and Walton) and tributary streams (Colne, Abbey River, and Wey) as well as from adjacent parts of the Thames.

(a) Consideration of individual tributaries and backwaters.

(i) *Athens backwater at Eton* (Table III). The bed of this backwater, which is rather shaded, was at that time overgrown with Angiospermous aquatics. The composition of its plankton did not differ appreciably from that of the main river, although some of the commoner diatoms (e.g. *Melosira varians*, *Fragilaria capucina*, *Synedra ulna*, *S. acus*, *Navicula cryptocephala*, *Gyrosigma attenuatum*) were rather more abundant. On the other hand, *Fragilaria pinnata*, which was common in the backwater, was rare in the river itself. This species is likewise often abundant in Shepperton backwater, though seldom common in the main river. There were no obvious differences in the composition of the plankton of the main river above and below the backwater.

(ii) *The River Colne* (Table II) at Staines, near its entry into the Thames, is very shallow, but at the time of examination the current was swifter than that of the main river. Probably in relation to this the plankton was quantitatively poorer than that of the Thames, although certain species, chiefly epiphytes (e.g. *Diatoma vulgare*, *Synedra ulna*, *Navicula cryptocephala* var. *intermedia*, *Rhoicosphenia curvata*, *Cymbella ventricosa*, and *Nitzschia amphibia*), were commoner. Certain minor differences in the composition of the plankton collected from the Thames above and below the point of inflow of this

TABLE II
The Phytoplankton of Tributaries of the Thames

	North Hinksey branch of Isis										Thame			Mole		Kennet		Wey		Thames above B-w. Bourne		Thames below Wey	
	8/8/1930	16/8/1930	1/8/1928	2/8/1928	15/8/1928	12/8/1928	16/8/1928	13/8/1928	13/8/1928	6/4/1929	25/5/1929	30/5/1929	Wey	Wey	Wey	Wey	Wey	Wey	Wey	Wey	Wey	Wey	
<i>Pandorina morum</i> .	SS	S S	—	R S	URU	FSC	USC	CFC	—	—	—	—	—	SSS	S	S	S	S	S	S	S		
<i>Pediastrum Boryanum</i> .	URU	USU	RIS	URR	UCR	URU	URU	SSS	—	—	—	—	—	URU	S	S	S	S	S	S	S		
" <i>duplex</i>	RUR	RUR	ASP	APP	PP	SSS	PFA	RIS	—	—	—	—	—	URU	S	S	S	S	S	S	S		
<i>Scenedesmus quadricauda</i>	—	—	—	I R	SCU	SCR	SS	URR	—	—	—	—	—	URU	S	S	S	S	S	S	S		
<i>Clotierium moniliferum</i> .	FRF	FUF	—	U F	RUR	SS	UFR	URR	—	—	—	—	—	—	—	—	—	—	—	—	—		
<i>Microcystis aeruginosa</i> .	—	PS	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
<i>Dinobryon divergens</i> .	—	—	—	—	S S	S S	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
<i>Melosira varians</i> .	CFF	PFP	FCC	UFF	SRS	FSF	SCU	FFC	—	—	—	—	—	ASP	S	S	S	S	S	S	S		
<i>Cyclotella Meneghiniana</i>	RS	—	RSS	SRS	CAP	RRU	CUF	SUR	—	—	—	—	—	URU	S	S	S	S	S	S	S		
<i>Stephanodiscus Hantzschii</i>	—	S S	—	—	SSR	SRS	SIS	SUR	—	—	—	—	—	PFP	S	S	S	S	S	S	S		
<i>Diatoma vulgare</i> .	UFU	USU	RAC	SSS	I	SSR	—	—	—	—	—	—	—	URU	S	S	S	S	S	S	S		
" <i>elongatum</i>	—	—	PPP	CUC	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
<i>Fragilaria capucina</i>	PUP	CSC	—	—	USR	CRC	URU	CRC	—	—	—	—	—	—	—	—	—	—	—	—	—		
<i>Asterionella gracillima</i> .	SUS	FUC	RUU	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
" <i>acuta</i> .	FCF	FFF	RUU	RUR	SRS	UCF	SRR	URU	—	—	—	—	—	—	—	—	—	—	—	—	—		
" <i>acuta</i> .	UUS	URS	SFU	SSS	SSR	RUR	SSS	RUR	—	—	—	—	—	—	—	—	—	—	—	—	—		
" <i>affinis</i>	—	CU	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
<i>Gyrodinium attenuatum</i> .	FRF	CRC	SSS	SSR	SRS	SRS	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
<i>Nitzschia cryptocephala</i> .	URF	PFC	SUS	FUF	UFU	FPF	UUU	RUR	—	—	—	—	—	—	—	—	—	—	—	—	—		
" (var. <i>intermedia</i>)	RPR	URU	—	RRS	R	SFU	SUR	SUS	—	—	—	—	—	—	—	—	—	—	—	—	—		
" <i>viridula</i> .	RUS	SRR	SS	SS	S I	R S	S S	SSS	—	—	—	—	—	—	—	—	—	—	—	—	—		
" <i>gracilis</i> .	UFU	FFF	SRR	UFU	RUR	RCU	RPC	FPF	—	—	—	—	—	—	—	—	—	—	—	—	—		
<i>Amphora ovalis</i> .	CFC	FUF	SRS	RFS	SSS	SRS	SCU	FCF	—	—	—	—	—	—	—	—	—	—	—	—	—		
<i>Nitzschia linearis</i> .	URS	SUR	SSS	SUS	SSS	U R	S S	RUR	—	—	—	—	—	—	—	—	—	—	—	—	—		
" <i>recta</i> .	URU	RCF	SUR	RUR	SSS	R R	SRS	UFR	—	—	—	—	—	—	—	—	—	—	—	—	—		
" <i>amphibia</i>	US	ICS	—	RI	—	—	FS	FAF	—	—	—	—	—	—	—	—	—	—	—	—	—		
" <i>communis</i> .	—	—	—	—	SRR	RCF	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		

Note:—First and last symbols in each column indicate frequency in Thames above and below tributary, respectively; that in italics of the tributary

tributary were almost entirely due to epiphytic species (e.g. *Cocconeis placentula*, *Cymbella prostrata*, *Nitzschia amphibia*, *N. acicularis*, *N. dissipata*, and *Cyclotella Meneghiniana*).

TABLE III
The Phytoplankton of Backwaters

	Athens B-w. Eton 8/8/1930	Chertsey 16/8/1930	Shepperton		'Sale' B-w. Walton 18/8/1930	Abingdon 2/8/1928
			9/8/1930	17/9/1932		
<i>Eudorina elegans</i>	—	RRR	R S	I	FS	SSI
<i>Pediastrum Boryanum</i>	UUU	URF	USU	USU	UUU	RRU
<i>duplex</i>	ISI	R/R	USR	USR	RRU	APA
<i>Closterium moniliferum</i>	FRF	FRF	CSC	FRF	FUU	SSU
<i>Merismopedia glauca</i>	—	S	—	—	US	—
<i>Synura uvela</i>	—	—	UI	RFR	S	—
<i>Melosira varians</i>	PAP	PRP	PSP	PUC	PFP	FUU
<i>Fragilaria capucina</i>	CPC	CRC	FUF	RUR	CPP	PCC
<i>pinnata</i>	UCU	S S	RAC	RAC	RCR	—
<i>Asterionella gracillima</i>	—	CUC	S S	S I	CFC	—
<i>Synedra ulna</i>	UFU	FFF	FFF	FRU	RUU	RRR
<i>acus</i>	RUR	SSR	UUR	SUR	RFR	SSS
<i>Gyrosigma attenuatum</i>	UFU	CUC	CRF	FRC	CSC	SSS
<i>Navicula cryptocephala</i>	FCF	CUC	FSU	FSU	CFC	SCF
" " var. <i>intermedia</i>	RRU	URF	S S	FRR	USU	RR
<i>Amphora ovalis</i>	FUF	FRU	UFF	URR	FFU	SSR
<i>Nitzschia linearis</i>	URS	RUS	UCF	FPF	RRU	SRS
<i>recta</i>	RSR	FUR	URR	USU	URR	RRR
<i>sigmoidea</i>	UUU	RFU	UCF	FFF	SRS	SSS
<i>acicularis</i>	—	CR	—	—	—	—

Note:—First and last symbols in each column indicate frequency in Thames above and below backwater, respectively; that in italics of the backwater.

(iii) The sluggish *Abbey River* near Chertsey (Table II) enters the Thames by means of a side-arm, into which part of the water of the main river is diverted over a weir. The Abbey River was both qualitatively and quantitatively richer in plankton than the adjacent part of the Thames. *Microcystis aeruginosa*, *Synedra affinis*, and *Nitzschia amphibia* were all common, although rare in the Thames below the point of inflow, while none of these species were present in the main stream above the entry of the Abbey River. It is clear that the three species mentioned above, which are rare in the main river, are supplied from this tributary stream.

(iv) *The backwater at Chertsey* (Table III), which enters the Thames just below the side-arm mentioned above, is very shallow and much shaded. Generally speaking, it had a poor plankton with *Nitzschia acicularis* as the most prominent species; this was absent from the Thames above Chertsey but occurred rarely in the plankton of the main river below the backwater. Green algae and most of the common diatoms (e.g. *Melosira varians*, *Fragilaria capucina*, *Asterionella gracillima*, *Gyrosigma attenuatum*, *Navicula gracilis*, *N. cryptocephala*, *Amphora ovalis*) were rare in the plankton of the backwater. *Nitzschia sigmoidea* was rather common though rare in the main river.

(v) *The backwater at Weybridge* (Table II) is formed by a loop into which

the river flows over a weir. The two branches of the Wey enter the lower end of this backwater on the south side just before it joins the main stream, while the Bourne, which is smaller and very shallow, enters the middle of the loop. Collections, made in the backwater above and below the entry of the Bourne, showed a phytoplankton in general similar to that of the Thames above the backwater, although below the point of entry of the Bourne *Asterionella gracillima*, *Cocconeis placentula*, *Navicula cryptocephala*, *Nitzschia recta*, and *N. sigmoidea* were commoner than above.

(vi) The plankton of the above-mentioned lower branch of the *River Wey* was poorer than that of either the backwater or the main river, many of the common diatoms (e.g. *Fragilaria capucina*, *Gyrosigma attenuatum*, *Navicula cryptocephala*, *Amphora ovalis*) being sparsely represented. On the other hand, *Microcystis aeruginosa*, *Navicula viridula*, and *Nitzschia linearis* were more abundant than in the main river; the first of these was slightly more common, while *N. viridula* was much more plentiful in the Thames below Shepperton Lock than in the backwater or in the Thames above that point (Table II).

It has already been pointed out (p. 564) that *Navicula viridula* is found in considerable quantity on the sandy bottom in shallow water below Shepperton Lock during the spring but not, as a rule, during summer. The relative abundance of this species in the Thames, below the entry of the Wey, in August is therefore probably due to contributions from the tributary.

Pediastrum Boryanum, *Fragilaria pinnata*, *Synedra ulna*, *Gyrosigma attenuatum*, *Nitzschia recta*, *N. sigmoidea*, *Cymatopleura solea*, *C. elliptica*, and *Suriella robusta* var. *splendida* were all commoner in the Thames, below the points of inflow of the Wey and of the backwater, than in the main stream above the weir at the head of the backwater. The greater frequency of *N. sigmoidea* and *N. recta* at this point may be due to contributions from the Bourne, while *S. robusta* var. *splendida* is no doubt contributed by the Wey.

(vii) *Shepperton Backwater* (Table III) is a long, shallow arm in part considerably overgrown with macrophytes and shaded by trees and shrubs. The plankton contained few green algae, and although the majority of the usual diatoms were present several species which were relatively common in the river (e.g. *Melosira varians*, *Gyrosigma attenuatum*, *Navicula cryptocephala*, and *N. viridula*) were sparsely represented. On the other hand, *Fragilaria pinnata* was abundant in the backwater and moderately so in the river below it, although rare above. *Cymbella Ehrenbergii* and *Synura uvella*, both rather common in the backwater, were practically unrepresented in the Thames. *Nitzschia linearis*, *N. sigmoidea*, and *Amphora ovalis* were commoner than in the main river and somewhat more plentiful below than above the backwater.

A further examination of this backwater was made two years later, in September, with somewhat similar results. *Fragilaria pinnata* was again commoner below the entry of the backwater. *Synura uvella*, *Nitzschia linearis*, *Synedra ulna* var. *biceps*, and *Cymbella Ehrenbergii* were all more frequent in

the backwater, the last being absent from the main river above the point of junction. On the other hand, the following diatoms, which were well represented in the plankton of the Thames, were poorly represented in the backwater: *Melosira varians*, *Stephanodiscus Hantzschii*, *Synedra ulna*, *Cocconeis placentula*, *Gyrosigma attenuatum*, and *Navicula cryptocephala*.

The amount of *Synura* present in this backwater, both in 1930 and 1932, was much greater than that encountered elsewhere at this time of the year, which may indicate that conditions were here more favourable for the development of this species than in the river itself. It is evident, too, that Shepperton backwater is a source of supply of *Fragilaria pinnata* to the main river, although it is by no means the only one, since this diatom was regularly found in the river above Shepperton and is also plentiful in the Athens backwater (p. 569). This species appears to thrive in the more sheltered backwaters.

(viii) The 'Sale' at Walton (Table III) is a broad backwater connected with the Thames just below Walton Bridge. There is scarcely any shading by trees or shrubs but the bed is somewhat overgrown with macrophytes. This was the only part of the river in which animals (Rotifers and Crustaceans) were at all common during the summer of 1930.

The plankton was both qualitatively and quantitatively richer than that of the Thames, and exhibited greater diversity than that of any other backwater or tributary examined, with the possible exception of Shepperton backwater. Green algae were as well represented as in the Thames itself, but *Eudorina elegans* was only found in the latter below the point of inflow of the 'Sale'. *Merismopedia glauca*, though considerably rarer, showed a similar distribution.

Of the diatoms *Cocconeis placentula*, *Synedra acus*, *Navicula radiosa*, *Cymbella helvetica*, *Gomphonema constrictum*, and *G. acuminatum* were all better represented in the 'Sale' than in the Thames. With the exception of *S. acus* and possibly *N. radiosa*, these are all undoubted epiphytes. On the other hand, *Melosira varians*, *Diatoma vulgare*, *Gyrosigma attenuatum*, and *Navicula viridula* were better represented in the main river than in the backwater.

Apart from the series of investigations just dealt with, a number of samples were also collected at various times from tributary streams and backwaters connected with other stretches of the river, with a view to ascertaining the possible effect of their plankton upon the composition of that of the main stream. Thus in August 1928 several tributary streams near Oxford were examined, viz. the Cherwell, the Ock, the North Hinksey branch of the Isis, and the Thame.

(ix) The bed of the *Cherwell* (Table II) was overgrown with a luxurious macrophytic vegetation such as had previously covered the bed of the adjacent Thames, and above the entrance of the Cherwell this growth was still in process of being cut. Green algae were practically absent from the Cherwell, but the diatom flora was similar to that of the Thames, except that *Diatoma elongatum*

and *Synedra acus*, both rare in the Thames above the point of inflow of the Cherwell, were well represented in the plankton of the latter and more frequent in the main stream below this point. *Melosira varians*, too, was common in the Cherwell and in the main river below its point of entry but was less frequent above.

(x) At *Abingdon* (Tables II and III) both the *backwater* and the *River Ock* were examined. The plankton of the backwater did not differ materially from that of the main river, except for the frequency of *Navicula cryptocephala*; this species, though very rare in the main river above the backwater, was rather common below it.

The part of the Ock near its inflow into the Thames is much overshadowed by buildings and trees. Although the Thames at this time contained a number of species of green algae, *Pediastrum* was alone represented in the Ock. The diatom flora was similar, but *Amphora ovalis*, *Nitzschia linearis*, and *N. sigmoides* were more abundant in the tributary.

(xi) A fortnight later collections were made from the *North Hinksey branch of the Isis* (Table II), which after flowing through flat, open country enters the main stream about a quarter of a mile below Osney Lock. The bed was much overgrown with aquatic macrophytes intermingled with *Cladophora*, which was densely covered with an almost pure growth of *Cyclotella Meneghiniana* (cf. p. 561). Despite the dense growth, the current was much faster than that of the Thames. The only difference in the plankton lay in the greater abundance of *Pediastrum Boryanum*, *Scenedesmus quadricauda*, *Cyclotella Meneghiniana*, and *Achnanthes* (? *minutissima*). Of them only *S. quadricauda* and *C. Meneghiniana* showed a greater frequency in the Thames below the point of junction.

An examination of the same stream at about the same time in the following year brought to light more numerous differences. *Pandorina morum*, *Fragilaria capucina*, and *Stephanodiscus Hantzschii*, though relatively common in the Thames, were rare or absent in the North Hinksey branch, while *Scenedesmus quadricauda* and *S. obliquus* were common in the latter but rare in the Thames. A number of diatoms were more numerous in the tributary, viz. *Cocconeis placentula*, *Navicula cryptocephala*, *N. gracilis*, *Cymbella cistula*, *C. affinis*, *Gomphonema constrictum*, and *Nitzschia communis*. The last named was much more abundant in the Thames below than above the tributary, while *Cymbella cistula*, though absent above, occurred in small numbers below the junction. Furthermore, *Cosmarium ornatum* was equally frequent in the tributary and in the Thames below, although practically absent above. There is some evidence, therefore, that this stream at this time of the year supplies the Thames with certain green algae as well as with *Cymbella cistula* and *Nitzschia communis*.

(xii) *The Thame* (Table II) below Dorchester, examined in August of 1928 and 1929, is shaded by willows, &c., and the bed bears a luxuriant growth of aquatics.

In August 1928 green algae were less numerous, while *Melosira varians*, *Cocconeis placentula*, *Navicula gracilis*, *N. menisculus*, *Amphora ovalis*, *Nitzschia hungarica*, and *N. amphibia* were all more abundant than in the Thames. The last-mentioned species was only found in the main river below the Thame, while *Navicula gracilis*, *Melosira varians*, and *Amphora ovalis* were commoner below the point of junction.

In August 1929 green algae were equally well represented in the tributary and in the Thames, although *Eudorina elegans*, *Scenedesmus quadricauda*, and *S. obliquus* occurred in the latter only below the point of junction. *Synura uvella* was commoner in the Thame, *Dinobryon divergens* more abundant in the Thames. There was a similar disparity between the diatom flora of tributary and main river as in the previous year, though not as marked; only a few species (*Navicula cryptocephala*, *Nitzschia linearis*) were more plentiful in the main river below the tributary.

In both years there is evidence that the Thame acts as a source of supply for certain species, but these differ in the two years.

During April and May of 1929 samples were taken from the rivers Wey, Ember (a branch of the Mole), and Kennet.

(xiii) The rather shallow *Ember* had a much poorer diatom flora, *Stephanodiscus Hantzschii*, *Diatoma vulgare*, *Fragilaria capucina*, *Asterionella gracillima*, *Synedra ulna*, and *Nitzschia linearis* being much rarer in the tributary. Only *Navicula viridula* and *Nitzschia acicularis* were more frequent, which is not surprising, since both species are known to occur abundantly on the bed in the shallower parts of other rivers (Butcher, 1932).

(xiv) The water of the *Kennet* was very dirty and appeared to be considerably polluted. It contained numerous clumps of *Oscillatoria limosa*. Except for the paucity of green algae and of *Dinobryon divergens*, the plankton did not differ materially from that of the Thames, although *Diatoma vulgare*, *Stephanodiscus Hantzschii*, *Navicula gracilis*, *Nitzschia recta*, and *N. acicularis* were commoner. *D. vulgare* and the two species of *Nitzschia* were more frequent in the main river below than above the point of inflow.

(xv) The *Wey* (Table II) was examined at Guildford, samples having been taken on the previous day from both below and above the point of entry of this tributary into the main stream. The few green algae and Chrysophyceae present were rare and the majority of diatoms encountered were far less abundant than in the Thames. The only exception was constituted by *Navicula viridula*, which, however, was more plentiful below than above the point of inflow of the Wey. Similar results were obtained when the Wey was examined at Weybridge in August 1930 (p. 572).

(b) *The influence of the phytoplankton of tributary streams and backwaters on that of the main stream.*

There is some evidence that *Navicula viridula* and certain of the rarer constituents of the Thames phytoplankton, such as *Eudorina elegans*, *Scenedesmus*

quadricauda, *S. obliquus*, *Microcystis aeruginosa*, *Merismopedia glauca*, *Diatoma elongatum*, *Fragilaria pinnata*, *Synedra affinis*, *Nitzschia amphibia*, *N. communis*, and *N. acicularis*, are supplied from backwaters or tributary streams.¹ Such sources of supply may also in part account for local differences in the character of the plankton (p. 568), although not affording a full explanation.

In many cases at least local abundance of a particular species or a group of species seems to be due to special local factors in the main river itself. These encourage the development of certain species, probably on a part of the river-bed or on the macrophytes inhabiting the slower-flowing water near the banks. It is noticeable that in numerous instances species are more abundant in the river than in the adjacent tributary or backwater, and in such cases one can only conclude that they are derived from higher stretches of the river where conditions suitable for growth and multiplication obtain that are not realized in side-arms. A striking feature, too, is the often-marked discrepancy between the plankton of the backwater or tributary and that of the adjacent parts of the main stream. A species abundant in the former may not uncommonly be rare or absent in the latter. This indicates that conditions of existence must often be markedly different in the main river and its side branches, and that a species which may prosper in the latter cannot survive when introduced into the former.

In a slow-flowing river such as the Thames the successful planktonts probably multiply in their passage downstream, as was observed in *Melosira varians*, *Synedra ulna*, *Pandorina morum*, and *Eudorina elegans*. A species, even if introduced in abundance from a side-arm, would appear rare in the main river unless such multiplication took place.

As regards the majority of the commoner species, and more especially the diatoms, there is no evidence that they are essentially recruited from the tributaries or backwaters. It is probable that in the Thames, as suggested by Butcher (1932) for other British rivers, the constituents of the plankton are largely derived from the bed in the shallower parts of the river. During the course of this work algal growth scraped from stones on the river-bed, from wooden piles, &c.² (Table IV) was occasionally examined. Such growth consisted almost entirely of diatoms, the large majority of which were regularly encountered in the plankton. Some of these are true epiphytes attached by one valve (*Cocconeis*, *Amphora*) or more loosely by mucilage, stalks, or pads (*Rhoicosphenia*, *Gomphonema*, *Cymbella*, *Achnanthes*, some species of *Synedra*, and possibly also *Diatoma*, *Fragilaria*, and *Melosira*). As Butcher, however, has pointed out, a large number of forms with no obvious means of attachment regularly occur in such growths; these include species of *Cyclotella*, *Navicula*, *Nitzschia*, and *Surirella*. Without further investigation no definite conclusions can be drawn from these random collections, but they

¹ See pp. 569 to 575.

² Cf. also pp. 561, 563, 564, and 565.

indicate that certain common plankton diatoms, without visible means of attachment, are on occasion also abundant on the river-bed.

It would appear from the data given on pages 568 and 569 and in the Table that a species, although multiplying actively in one part of the river, may cease

TABLE IV
The Microflora of the River Bed

	Kew			Shepperton	
	28/5/28	7/9/28	29/6/30	30/5/29	19/3/32
<i>Melosira varians</i> . . .	UC	RU	RF		
<i>Cyclotella Meneghiniana</i> . . .	RS	UC	RU		AF
" <i>Kutzingiana</i> . . .			RS		
<i>Stephanodiscus Hantzschii</i> . . .		FC	CF		
<i>Thalassiosira decipiens</i> . . .	SS	RF	RS		
<i>Diatoma vulgare</i> . . .	UF	RR		CR	AC
<i>Fragilaria capucina</i> . . .					CR
<i>Synedra ulna</i> . . .	RF	CU		FU	PP
" <i>acus</i> . . .				RS	FR
" <i>affinis</i> . . .	US				
<i>Achnanthes lanceolata</i> . . .	F	R			
" <i>linearis</i> . . .	A				
" <i>exigua</i> . . .		U			
<i>Cocconeis placentula</i> . . .		RU	RR		RR
<i>Rhoicosphenia curvata</i> . . .		CR	UU	US	SS
<i>Navicula mutica</i> . . .		RR	RS		
" <i>cryptocephala</i> . . .	US	RF	SR		
" (var. <i>intermedia</i>) . . .		UR	RR		
" <i>viridula</i> . . .	RP		SF	FS	PC
" <i>gracilis</i> . . .	RF	CU	SR	RU	RR
<i>Amphora ovalis</i> . . .		UR	RR		S
" var. <i>pediculus</i> . . .		P	C		
<i>Cymbella prostrata</i> . . .					U
<i>Gomphonema parvulum</i> . . .			F		
" <i>olivaceum</i> . . .		P	FS	P	R
<i>Nitzschia linearis</i> . . .	SP			RF	UC
" <i>amphibia</i> . . .	FS	CR	R		
" <i>fonticola</i> . . .	F				
<i>Surirella ovata</i> . . .	FU	RS	UU	CR	FF

Note:—Letters in italic indicate frequency of these organisms in the plankton.

to do so in a lower stretch. This seems to be the most natural explanation for abundance of a species at one point and great scarcity at another. How rapidly a species can disappear, when conditions for its existence are not suitable in the main river, is illustrated by the frequent marked discrepancy between the plankton of a backwater or tributary, and the part of the main river below its point of junction.

V. THE EFFECT OF THE TIDE UPON THE COMPOSITION OF THE PLANKTON AT KEW

This was investigated on two separate occasions by collecting samples at Kew at three-hourly intervals, at different states of the tide (Table V). In August 1929 there were few striking differences in the composition of the

plankton at full tide, half ebb, low tide, and half flood. At high tide, however, *Coscinodiscus excentricus* was abundant and *Fragilaria crotonensis* rather common, whereas both were much rarer at all other times. *Cyclotella Meneghiniana* was common only at low tide. In August 1930 there was even less contrast,

TABLE V
Kew—Tidal Effect

	August 1929				August 1930		
	Low.	Half flood.	High.	Half ebb.	High.	Half ebb.	Low.
<i>Ceratium hirundinella</i>	—	—	—	—	F	U	F
<i>Melosira varians</i>	U	R	R	R	R	U	U
„ <i>granulata</i>	R	S	R	S	F	C	F
<i>Cyclotella Kutzingiana</i>	U	F	U	F	—	S	S
„ <i>Meneghiniana</i>	F	R	R	R	R	S	U
<i>Coscinodiscus excentricus</i>	U	U	A	U	S	S	S
<i>Thalassiosira decipiens</i>	C	C	F	C	U	U	U
<i>Biddulphia aurita</i>	S	S	R	R	I	S	S
<i>Fragilaria crotonensis</i>	R	R	F	R	A	A	P
<i>Rhaphoneis amphiceros</i>	R	U	U	U	R	S	S
<i>Asterionella gracillima</i>	S	S	S	S	C	F	C
<i>Synedra ulna</i>	F	U	R	U	R	R	R
„ <i>acus</i> var. <i>angustissima</i>	F	R	U	U	—	—	R
<i>Navicula cryptocephala</i>	S	S	—	S	U	U	F
„ <i>viridula</i>	R	R	R	U	R	R	F
<i>Nitzschia sigma</i>	U	U	U	F	U	R	S

the only notable difference being the greater abundance of *Navicula viridula* at low tide. It seems, therefore, that at Kew, which is almost at the limit of tidal influence, the tide exerts very little effect upon the plankton during the course of a day, except that upon occasion large numbers of truly marine species, such as *Coscinodiscus excentricus*, may be temporarily introduced.

The majority of the marine diatoms occurring in the Kew plankton (i.e. *Thalassiosira decipiens*, *Rhaphoneis amphiceros*, *Biddulphia aurita*, and *Nitzschia sigma*) appear to be equally common at all states of the tide, but are seldom found in a healthy state. In this connexion it may be noted that the essentially marine *Bacillaria paradoxa*, already recorded in higher reaches by Fritsch (1902 and 1903), was found on several occasions in small numbers but in quite a healthy condition as far up as Shepperton. At Kew there is little variation in salinity during the course of a day.

Mr. Stephenson informs me that in the lower stretches the salinity varies much more markedly with the seasons than with the state of the tide, the difference being almost entirely due to rainfall. For example, at Barking the salinity may be as low as 6.6 parts of sodium chloride per 100,000 (= 4 parts of chlorine per 100,000) after a spell of wet weather, whereas in dry weather it may be as high as 1,430 parts of sodium chloride per 100,000 (= 868 parts of chlorine per 100,000). The salinity was unusually high in the summer of 1929, no doubt as the result of the lack of rainfall.

A study of the plankton at Kew, especially in the summer of 1929 with this high salinity, shows that many of the freshwater forms can tolerate a greater salt concentration than is normally present above the tidal zone, without showing signs of damage. On the other hand, certain species (e.g. *Pandorina morum*, *Melosira varians*, *Fragilaria capucina*, *Navicula cryptocephala*, and *Nitzschia sigmaidea*) which were common at Shepperton during the summer of 1929 were less frequent at Kew. Others which play a smaller role in the plankton of the river (viz. *Pediastrum duplex*, *P. Boryanum*, *Closterium moniliferum*, *Dinobryon divergens*, *Diatoma vulgare*, *Gyrosigma attenuatum*, and *Nitzschia sigmaidea*) were always less common at Kew than at Shepperton. Their scarcity cannot, however, with certainty be attributed to the higher proportion of salts present. Two other important factors at least, namely, a low percentage of aeration and the presence of a large amount of organic matter, may have been responsible.

It must also be borne in mind that the presence of chlorides (chiefly sodium chloride) is not wholly due to the influx of tidal water, but is also, at least in fresh waters, an indication of pollution by sewage effluents.

In order to avoid the occasional tidal effect, the periodic collections of material at Kew were always made at or about low tide. This was also advantageous in that there was no disturbing wash from tugs or river steamers.

VI. GENERAL SUMMARY OF PARTS I AND II

The phytoplankton of the Thames, like that of other rivers, consists very largely of diatoms and exhibits a well-marked seasonal sequence. In January *Asterionella gracillima* is the commonest species, while *Synura uvella* becomes prominent in February. The maximum plankton content is realized in March, the dominant species being *Nitzschia linearis*, *Surirella ovata*, *Synedra ulna*, and *Navicula viridula*, while *Asterionella*, *Synura*, and *Melosira varians* are often plentiful. In April *Nitzschia*, *Melosira*, and *Navicula* are still plentiful, but *Synedra*, *Surirella*, and *Synura* decrease in amount, while *Diatoma vulgare* comes to the front. In May *Melosira varians* is the dominant, while *Nitzschia linearis* and *Navicula viridula* have decreased in number. *Asterionella* may still be prominent. Green algae appear in this month especially at Shepperton, to become more prominent in June. In the latter month *Stephanodiscus Hantzschii* is often the most frequent species and continues to be common in July; at Shepperton *Melosira varians* is sometimes equally abundant.

The most marked difference in the plankton at Kew and Shepperton is observed in August and the following months. This is probably due to the stronger influence of pollution or influx of salt water at Kew owing to the scarcity of flood water. At Shepperton in August *Melosira varians*, *Navicula cryptocephala*, *N. viridula*, and *Synedra ulna* all occur in moderate numbers, while at Kew the commonest species are *Thalassiosira decipiens*, *Melosira granulata*, and *Cyclotella Meneghiniana*. In September *Melosira varians*, *Navicula cryptocephala*, and *Gyrosigma attenuatum* are common at Shepperton,

while at Kew the most prominent species are *M. granulata* and *T. decipiens*. Green algae are now becoming rare. In October *Melosira varians* is plentiful at Shepperton while at Kew *T. decipiens* is still common and *M. granulata* and *Asterionella* frequent. In November the difference between the plankton at Kew and Shepperton disappears. *M. varians* is still common with *Gyrosigma attenuatum* well in evidence.

A definite relation to a falling water-level is evident, the amount of phytoplankton being greatest some time after the strength of the current has begun to decrease in the early months of the year. The vernal diatom maximum is always associated with a high percentage of nitrate. The frequencies of many of the commoner species of the phytoplankton appear to be definitely related to certain environmental factors, the data in question having been summarized on page 567.

The composition of the plankton in the different reaches of the river, though in general of a uniform character, may show marked variations. This has been established for the summer months, but is likely to be true also at other times of the year. The plankton of a considerable number of backwaters and tributaries has been examined collaterally with that of the main stream above and below the point of junction. These investigations show that, whilst in a few cases local differences in the plankton of the Thames can be ascribed to the introduction of material from such a source, this explanation by no means applies to all cases. Local abundance appears often to be due to local factors favouring the development of certain species, either on the river-bed or on macrophytes growing in shallower water near the banks.

The investigations just mentioned afford little evidence in favour of the view expressed by Fritsch and others that the plankton of such a stream is derived from that of backwaters and tributaries. Only in the case of some of the rarer constituents was there an indication that the free-floating microflora is recruited from side-arms of the river. It therefore seems probable that, as Butcher has already suggested, the bulk of the plankton is derived from the bed in the shallow parts of the river. Random collections from the river-bed showed that many of the diatoms found commonly in the plankton are on occasion also prominent members of the attached flora of the river. At the same time there is distinct evidence that many of the plankton may undergo considerable multiplication during their passage down stream and that local frequency may in some cases be due rather to this cause than to abundant supply from the source.

VII. ACKNOWLEDGEMENTS

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and in particular would mention the London County Council and Mr. Coste, their Chief Chemist; the late Sir Alexander Houston of the Metropolitan Water Board; the Thames Conservancy Board; and Mr. Stephenson, Consultant Chemist to the Port of London Authority. My thanks are also due to Mr. D. J. Scourfield for identifying certain prominent members of the zooplankton and to Dr. D. McCall for naming certain critical diatoms.

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Note:—Symbols in *italic* indicate frequency at Shepperton. Symbols in ordinary type indicate frequency at Kew.

[illegible]

Conjugation and Zygote Germination in *Allomyces arbuscula*¹

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With Plates XVIII to XXII and thirteen Figures in the Text.

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I. INTRODUCTION

WHILE the sexuality of *Allomyces* (*A. javanicus* and *A. arbuscula*) has received considerable attention in mycological circles it deserves wider recognition, first, because it illustrates a very interesting type of sexuality and, second, because it presents material by which our knowledge of sexual phenomena can certainly be advanced. Concerning its type of sexuality it must suffice to say that it represents the only instance of heterogamous, planogametic conjugation known to exist in the fungi. Of those attributes of its sexuality that particularly recommend it as good experimental material the following seem most significant:

First, these fungi are easily cultured, either on agar or in water.

Second, all aspects of sexuality, i.e. gametogenesis (sex segregation and expression), conjugation, and zygote germination, can be profitably studied in the living condition, even under the highest powers of the microscope.

Third, this chain of sexual manifestations from gametogenesis to zygote germination can be followed at one sitting. Gametogenesis, from the gamete origin stage (the usual point of departure in such studies) to the discharge of gametes, takes about forty-five minutes. Conjugation usually occurs within the next ten to twenty minutes and zygote germination follows in one and one-half to two and one-half hours. Thus, the whole process of sexuality,

¹ This work was done while holding a National Research Fellowship at Harvard University.
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usually so difficult to observe in its entirety, passes in review in less than four hours.

Fourth, the expression of sexuality in *Allomyces* is most spectacular. For instance, the male and female gametangia, in addition to differences in size, shape, and position, also differ in colour, the male being orange, the female grey. Between the gametes likewise there is a similar if less extreme colour distinction in that the male is brassy, the female grey. But this is not the only distinction between gametes, for here, as in the gametangia, there are other differences, differences in size, motility, and internal constitution that offer equally good, if less marked criteria of sex.

Finally, it is to be noted that the gametes are uninucleate. The advantages of this condition in any cytogenetic study should not be overlooked.

Such a promising and altogether remarkable type of sexuality deserves careful analysis. To this end a cytological investigation was inaugurated into the processes of gametogenesis, conjugation, and zygote germination. The results of the studies on gametogenesis having already been reported (Hatch, 1935) we now have only to consider conjugation and zygote germination.

II. CYTOLOGICAL METHODS AND TECHNIQUES

A. Methods.

To obtain gametes, conjugants, and zygotes in quantity, and to provide an easy means of handling them during fixation, staining, and mounting, the following simple procedure was followed:

1. Sexual mycelia were obtained as described in a previous paper (Hatch, 1935, p. 625).
2. When three days old these mycelia were transferred to sterile, 5-cm. Petri dishes filled with sterile, distilled water, on the bottom of which was placed a sterile cover-slip.
3. The mycelium was suspended over the cover-slip and left in that position for one to four hours, at the end of which time it was removed. By this time the cover-slip had acquired a rich flora of gametes and conjugants and some germinating zygotes, for from the moment the mycelium was first suspended over it a steady fall of gametes and zygotes had rained down on the cover-slip.
4. The water was then drained off and the fixative introduced. (To study *gametes* fix from one to two hours; *conjugants* from two to three; *zygote germlings* from three to four. To trace zygote germination progressively postpone fixation.)
5. Since the gametes and zygotes fixed themselves to the cover-slip they could be washed, stained, destained when necessary, dehydrated and mounted in balsam by carrying the cover-slip through the requisite changes and ultimately mounting it.

B. Techniques.

1. For gametes, conjugants, and planozygotes.
Fixative: A modified Schaudinn's fluid ($\frac{1}{3}$ strength)+2 per cent. acetic acid (15 minutes).
Stain: iron-alum haematoxylin.¹
2. For germinating zygotes and for nuclei and nuclear detail.
Fixative: $\frac{1}{3}$ per cent. solution of mercuric chloride+2 per cent. acetic acid (15-30 minutes).
Stain: iron-alum haematoxylin.¹
3. For cytoplasm.
 - a. Nuclear caps—Meves and Champy-Kull.
 - b. Lipoid granules—osmic vapour, and iron-alum haematoxylin.¹

III. THE GAMETES

The male and female gametes of *A. arbuscula* are both of the same shape, both show precisely the same type of ciliation, and both are alike in their internal organization. The differences, obvious and striking though they are, are not differences in kind but differences in proportions and pigmentation. There is, accordingly, no need of a separate discussion of both gametes. A careful description of one gamete, the female, should suffice if we reserve for the other a consideration of the relative proportions and differences in pigmentation.

The female gamete.

The female gamete is a spherical to subglobose cell approximately $11\ \mu$ in diameter (Pl. XVIII, Figs. 1 and 5). It bears either one (Pl. XVIII, Fig. 1) or two (Pl. XVIII, Fig. 5) long, posteriorly-attached cilia. While the great majority of gametes are uniciliate, biciliate gametes are regularly found and these gametes, apparently, are quite as normal as those with but a single cilium (p. 586).

The gametes are uninucleate. The nucleus, however, is so completely invested by a bulky, more deeply staining, hence more prominent, nuclear cap that its separate identity might easily be overlooked. Improperly identified, the nucleus appears to attain extraordinary proportions. Properly identified, it is normal, for it is approximately only $3.6\ \mu$ in diameter. Its position near the posterior membrane, just opposite the point of cilium insertion, is explained by the fact that the nucleus is attached to the cilium. Springing from the nuclear membrane and extending to the point of cilium insertion on the gamete membrane is a thin, thread-like rhizoplast (Pl. XVIII, Figs. 1 and 7). While the primary importance of this connexion probably lies in its integrating function in ciliary motion, it also serves to anchor the nucleus to the gamete membrane. Its importance in this respect can be easily demonstrated, for when this connexion is broken, as is occasionally the case in violent

¹ Destained with saturated aqueous solution of picric acid (30 min.)

amoeboid movements, the nucleus falls away from the membrane (Pl. XVIII, Fig. 3). A second effect of the rhizoplast connexion upon the nucleus, an effect implied in its anchoring function, is the extension of the nuclear membrane when, as in ciliary activity, the rhizoplast is under tension (Pl. XVIII, Fig. 6, a male gamete).

The nucleus always contains a single, conspicuous nucleolus (Pl. XVIII, Fig. 1), which structure is as constant in size from gamete to gamete as is the nucleus itself. Its position in the nucleus is precisely the same as that of the nucleus in the gamete, and the explanation is also the same, for just as a rhizoplast anchors the nucleus close to the point of cilia attachment, so does an intranuclear extension of the rhizoplast draw the nucleolus close to the point of rhizoplast insertion (Pl. XVIII, Figs. 1 and 7). Furthermore, just as the rhizoplast occasionally effects a distension of the nuclear membrane, so does the intranuclear rhizoplast extension effect an extension of the nucleolar membrane. In the case of uniciliate gametes with one rhizoplast and with but a single intranuclear rhizoplast extension the nucleolus is drawn out into a conical shape (Pl. XVIII, Figs. 1, 13, et al.). With biciliate gametes, with two rhizoplasts and two intranuclear rhizoplast extensions inserted upon the nucleolus at two different points, the nucleolus has two arms and is, consequently, roughly crescent-shaped (Pl. XVIII, Figs. 5, a female, and 6, a male gamete). The true motor apparatus, then, consists of a long cilium inserted on the gamete membrane but continuous through to the nucleolus within the nuclear membrane by means of a rhizoplast and an intranuclear rhizoplast extension. The presence of a definite connexion between cilium and nucleolus suggests that the nucleolus may have a functional significance, may even play an important role in the ontogeny of the motor apparatus. It may or may not be pertinent to this discussion, but it certainly should be noted that in living gametes the nucleolus exhibits remarkable displacements, swinging now to the right, now to the left, plunging ahead or settling back in the nucleus.

The chromatin reticulum in which the nucleolus is embedded gives the appearance of being centred upon the nucleolus by reason of the slightly heavier strands that radiate from that body. The reticulum of the female gamete is characteristically looser than that of the male.

In the female gamete, as has already been mentioned, the nucleus is not the most prominent protoplasmic inclusion. More prominent is the nuclear cap, a larger, more deeply staining, cytoplasmic body (Pl. XVIII, Figs. 1, 2, and 8). This remarkable structure invests the nucleus over more than half of its anterior surface and, as its name implies, is drawn down over the nucleus like a cap or, more properly, like a busby. The union between the nucleus and the nuclear cap is intimate and the two, especially in their early association, appear and move more as a single structure than as separate ones, i.e. any displacement of the one results in a like displacement of the other.

While it must already be apparent that the nuclear cap is an anomalous structure this can be appreciated only when its transitory nature is taken

into consideration. It is a structure found only in the motile cells of *Allomyces*, the male and female gametes and the zoospores. In the sexual mycelium, even in the hyphal tips from which gametangia are abjoined, no nuclear caps are to be observed. Nor are they evident in the gametangia themselves until some time after the gametes have separated out and dehiscence is imminent. But a nuclear cap is a most prominent structure in gametes. Whence does it come? The answer is to be found in a study of gametogenesis (or zoosporogenesis), where it appears that the nuclear cap is formed by the aggregation and coalescence of chondriosomes (Hatch, 1935). Still quite as baffling as its origin is its mysterious disappearance. In the zygote soon after conjugation the nuclear cap is a prominent structure but before germination this structure fragments and disappears not to be seen again until the asexual mycelium produces zoospores, the sexual mycelium gametes. In all probability it is converted back to chondriosomes (pp. 602 and 603). There is a third question, however, that we must answer at this point and that is what, precisely, is the structure of the nuclear cap?

Since the nuclear cap is apparently a chondriosomal complex the only accurate picture of its structure is to be gained from techniques designed to preserve cytoplasmic structures in general, chondriosomes in particular. But even with these techniques it was thought advisable to employ enough, five or six, so that a comparative study could be made of the several fixation patterns. Other evidence, unimpeachable as far as it goes, may be obtained from a study of the nuclear cap in living gametes. In living material the appearance of the nuclear cap can best be described as cloudy, if by 'cloudy' one visualizes a more or less translucent, mottled, turbidity in which a fundamental alveolar structure is suggested (Pl. XVIII, Fig. 8). This indication of an alveolar structure is apparent only in the body of the cap. Towards the periphery it is more dense and consolidated. With this picture in mind let us now consider and evaluate the evidence presented in nuclear caps fixed and stained first, by the method of Champy-Kull, second by that of Meves, these being the most successfully employed cytoplasmic techniques.

With Champy-Kull the nuclear cap proves to be a chambered structure bounded by a ragged, fissured envelope or shell, irregular and variable as to thickness (Pl. XVIII, Fig. 9, a male gamete). This shell and the walls of the chambers represent the chromatic material in the complex and stain a deep red, which colour, with this technique, indicates a chondriosomal affinity. In section the cap appears reticulate, its reticulum being characteristically swollen at its intersections. The stain, as would be expected, is taken up primarily by this reticulum, for it represents the disposition of the chromatic material in this view.

With Meves the nuclear cap shows the same structure, a ragged, fissured envelope and a chambered interior (Pl. XVIII, Fig. 10). It also shows the same staining reactions in that the chondriosomal stain, a deep grey-black in this case, is taken up by the same chromatic material. With this technique,

however, a more detailed analysis can be made of the precise organization of the cap, since here the structure of the chromatic material has apparently been more faithfully preserved. With Meves, while the organization of the chromatic material in some nuclear caps is precisely the same as that revealed in Champy-Kull preparations, in others it is differently distributed so that, though still chambered in effect, it gives to the nuclear cap an alveolar appearance. In still other nuclear caps it is so disposed that the cap takes on a structure that can best be likened to an aggregation of granules bound together by membranes, in section a knotted reticulum.

An explanation of this varied disposition of the chromatic material is not difficult to find if we recall that the nuclear cap is an aggregation of chondriosomes, structures which are known to have the capacity of vesiculation. But before concluding that the nuclear cap is an aggregation of vesiculated chondriosomes it would be well to consider whether the vesiculations of chondriosomes could produce a structure as intricate and variable as the nuclear cap. It appears that it could, for in the vesiculation of chondriosomes:

- (1) A vesicle of varying proportions may be formed upon the chondriosome. This would account for the looseness or compactness of the reticulum.
- (2) The membrane or envelope about the vesicle is chromatic, like the body of the chondriosome from which it is derived; the lumen is achromatic. This disposition of the chromatic and achromatic substances would result in a chambered structure if and when numerous vesicles were compressed together.
- (3) The thickness of the membrane is variable from time to time and place to place, depending upon the extent to which the substance of the chondriosome is dispersed over the membrane. This feature would account for the varied disposition of the chromatic material noted in the nuclear cap.

Other cytoplasmic inclusions of importance in the gametes of *Allomyces arbuscula* are the lipid granules. These are small spherical structures in constant Brownian movement. In their distribution they are somewhat restricted in that they scarcely ever take up either an extreme parietal or an extreme central position (Pl. XVIII, Figs. 8, 10, and 11). Theirs is the middle ground. In this distribution they follow the concentration of the undifferentiated cytoplasm which is vacuolate parietally, next to the membrane and centrally about the nucleus and nuclear cap. In fixed material the distribution of the lipid granules is very useful in determining the condition of the gamete* at the time of its fixation. If it is living the lipid granules will retain their median distribution; if dead they will be parietally disposed. This obtains from the fact that at death the lipid granules pass to the periphery of the gamete. In their activity, too, the lipid granules are good criteria of the well-being of the cell, for while their Brownian movement is

restrained in healthy gametes it is immeasurably more violent in dead and dying gametes. In addition to their limited Brownian movement the lipid granules are moved about from place to place by the movements of the gamete. When the gamete is swimming they usually become aggregated towards the anterior end of the cell. With the cessation of active motion, at least some of the lipid granules glide back around the nuclear cap and even gather behind the nucleus.

A very considerable significance is attached to these lipid granules because their pigmentation is an accurate indication of the sex of the gamete. In the female gamete they are dark grey, in the male, orange. Going back through gametogenesis for an explanation of this pigmentation we find that when the two gametangia, the male and the female, are first cut off from the hypha their lipid granules are dark grey. In the female gametangium no change occurs in the colour of the lipid granules, but in the male gametangium there is a decided change, for even before the gametes become delimited the lipid granules become orange. While this is one of the sharpest expressions of sex that we have and one that might lend itself most profitably to physiological and biochemical analysis, such a study has yet to be made.

But are these granules correctly named? Are they of lipid nature? On this point the evidence is largely negative in that while it can apparently be shown that these granules have neither the properties of true chondriosomes or true fats, their lipid nature is not proved. For example, with Champy-Kull, a differential cytoplasmic stain that is supposed to stain chondriosomes red and fats black, these lipid granules do not stain at all (Pl. XVIII, Fig. 9). With Meves, where the chondriosomes should theoretically stain a dark grey or black and fats black, the lipid granules stain a light brown (Pl. XVIII, Fig. 10). That they are closely related to fats—possibly possess some fatty component—is suggested by the fact that with Cramer's osmic vapour method where intense blackening indicates fat, they stain more deeply than the chondriosomal nuclear caps (Pl. XVIII, Fig. 11).

The male gamete.

The male gamete differs from the female in that it is only 8μ while the female is 11μ in diameter (cf. Pl. XVIII, Figs. 1 and 2). Its nucleus is proportionately smaller, being but 2.3μ , while that of the female is 3.6μ in diameter (cf. Pl. XVIII, Figs. 1 and 2). The same disproportion also holds between its nuclear cap and that of the female (cf. Pl. XVIII, Figs. 1 and 2). Finally, as we have already noted, its lipid granules are orange, while those of the female are grey.

The amoeboid activity of gametes.

While the manifestation of amoeboid activity by a gamete is dependent upon the age of the culture and the conditions of the environment into which the gamete is set free, I am not prepared, in this paper, to examine cause and effect any more than is necessary to select what appear to be instances of

normal gamete activity. Given a clean, young, aqueous culture in which gametangia are forming and dehiscing freely, we find that the female gametes exhibit their amoeboid behaviour at two different times. As soon as they are individualized after dehiscence they show restrained amoeboid movement (p. 591). This, however, persists for only a limited time, until conjugation supervenes or until the gametes display their ciliary activity and swim off. The second period of amoeboid activity occurs when the gametes abandon their ciliary activity and drop to the substratum. The amoeboid movements of the gametes during this period are unrestrained and vigorous. The amoeboid activity of male gametes differs from that of the female in only one respect; the first period of amoeboid activity is more often dropped out of their swarming cycle. The role of amoeboid movement in conjugation will be described later.

Observations on living amoeboids reveal that pseudopod extension is initiated by the formation of a hyaline bulge or blister on the surface of the gamete. With the subsequent extension of the pseudopod this hyaline area is carried up on its tip as a lenticular cap. Crowding in behind this cap are numerous lipid granules brought there by the concentration of the cytoplasm. Behind this area, more or less wedged in the base of the pseudopod, is the nuclear cap. Its precise disposition in the pseudopod can not be determined without fixing and staining the gametes. When this is done (Pl. XVIII, Figs. 3 and 4, 12-15) it appears that the nuclear cap suffers serious and often bizarre dislocations. In any really vigorous pseudopodial activity at least a portion of its substance is almost always drawn up or swept up into the pseudopod (Pl. XVIII, Figs. 3 and 4, 13-15). Very often the greater bulk of the nuclear cap is displaced from about the nucleus and deposited in a pseudopod, leaving behind on the nuclear membrane only scattered remains of cap substance (Pl. XVIII, Fig. 15). Sometimes all connexion is lost between these masses (Pl. XVIII, Fig. 14), sometimes, as in Pl. XVIII, Figs. 4, 13, and 15, this connexion is retained. From its susceptibility to distortion in the later stages of amoeboid activity, the nuclear cap appears to be made up of a rather malleable material. With the cytoplasm streaming towards the base and up into the pseudopod the gamete becomes vacuolate elsewhere, and this becomes particularly marked in those limits of the cell most distant from the pseudopod.

IV. CONJUGATION

Conjugation in *Allomyces* has been described by two different workers, but neither attempted to analyse the phenomenon very critically. Kneip (1929), working with *A. javanicus*, discovered zygotes in cytological preparations in which the progress of a nuclear and cytoplasmic fusion could be traced, but he never saw the fusion of a male and female gamete and never, apparently, made any direct observations on conjugation as it occurs in living material. The writer, in a preliminary paper (1933), described the process of conjugation

in *A. arbuscula*, but that account, based on observations of living material, was not complete and was not supplemented by a cytological study.

1. *Observations on living material.*

In this account of conjugation the arrangement of the gametangia and their time of dehiscence must be mentioned because they seriously affect the number of conjugations achieved. In *A. arbuscula* the male and female gametangia are formed in couplets or in chains in which the male and female gametangia regularly alternate. The advantage of this arrangement in increasing the frequency with which male and female gametes are brought in contact is obvious, provided, of course, that the gametes from contiguous male and female gametangia are discharged at the same time. This is precisely what happens in most instances.

But much of the advantage inherent in their arrangement and simultaneous discharge might well be lost if the gametes simply poured out of the emergence papillae and moved away. This does not happen. In the dehiscence of the female gametangium the gametes emerge slowly, often in large blobs so that they are not completely individualized when they first appear. But even when free and completely individualized they are slow to exercise their ciliary powers, hanging motionless, rocking gently as they seek to disengage their cilia, or, if quite free, crawling slowly about over the substratum. In a word, for one cause or another, they linger about the parent gametangium for a comparatively long time. The emergence of the male gametes is very different in that they slip out of their gametangium wholly individualized and immediately become active. If they begin their ciliary activities, as is usually the case, they explore their environment in short, wild dashes that carry some of them in among the more widely dispersed female gametes. If their motion is at first only amoeboid they crawl up over the male gametangium and eventually some attach themselves to the female gametangium, where they tend to mingle with the female amoeboids creeping down over its surface. Thus, dehiscence in its effective utilization of a fortuitous gametangial arrangement achieves a close proximity for male and female gametes. Sexual attraction, significantly enough, need not and cannot, apparently, be invoked to explain the achievement of this propinquity. For instance, it would be very difficult indeed to demonstrate that the female gametes possess any long-distance attraction for the free-swimming males. In the movements of amoeboid males a sexual response might at first seem indicated but this, I think, is more apparent than real and could be adequately and more correctly accounted for by accidental and more or less casual causes. Since these amoeboids spread, i.e. since they move away from the point of their emergence, and since their spread can proceed only in two directions, up or down the gametangium, on purely contingent grounds, on the average, one-half the number would move in the direction of the female gametangium. Further, since the emergence papillae of most male gametangia are distally located and discharge

their gametes about the base of the female is it not to be expected that more male gametes should crawl up on the female gametangium than creep down over the hypha? Thus, without resorting to any explanation involving sexual attraction, we can easily account for all the male gametes found on the female gametangium. Further, since there is always some downward spread, some drift away from the female gametangium, the activities of male amoeboids would seem to be primarily dictated by chance. That the female amoeboids creep towards the male gametangium is not significant either, for this is the only direction in which they could move if they used the female gametangium as a substratum. I think we must accordingly conclude that the final propinquity of male and female gametes is probably achieved by chance. If this be true, fortuitous gametangial arrangement and simultaneity of dehiscence attain real significance, for together they achieve in this fungus a high degree of sexual union.

While an effective long-range sexual attraction would seem to be lacking in *A. arbuscula*, a limited sexual attraction may exist. This attraction, if such it be, is only to be observed after a certain degree of propinquity has been established. An instance, and about the only instance I have seen of what might be called sexual attraction, was observed when a young and active male dashed into the centre of a group of three female gametes arranged as at the apices of an equilateral triangle, and forthwith ceased further movement. Before the advent of the male the females had been resting quietly. They were spherical and showed no reaction towards each other. As soon as the male appeared they threw out small pseudopods in its direction and began an unhurried march towards it. The first to make contact with the male fused with it, the male being passive.

From what has already been said about dehiscence it must be apparent that the place at which most male and female gametes are thrown into the closest relationship is about the tip of the female gametangium. Quite as obviously the time at which most gametes attain this advantageous distribution follows almost immediately upon dehiscence and persists for only some fifteen to twenty minutes, or until most of the gametes have left the gametangia. This is, then, the place and the time at which conjugation can be expected to take place, and so it does in large numbers. As a matter of fact, probably all but a small percentage of the total number of conjugations are achieved at this time. I say this more or less confidently because two things happen after active swarming begins that militate against successful conjugation. First, the greater dispersion of gametes makes the attainment of the requisite propinquity more difficult. Second, the ageing of the gametes makes the achievement of a successful conjugation more and more uncertain. That ageing is an important consideration in conjugation is evidenced by the fact that while conjugation in young gametes (one to fifteen minutes old¹) is quick, almost instantaneous, in older gametes (fifteen minutes or over) it becomes an

¹ The age of a gamete is computed from the time of initial dehiscence.

increasingly slower process until a point is reached where conjugation is actually impossible. Instances of failure in conjugation are numerous and conspicuous in all cultures. Time after time male and female gametes can be seen to come in contact and to glide continuously over each other, but without result. Since instances of this sort are to be found most regularly far from any gametangia, in regions that could be reached only after prolonged ciliary activity, the assumption is that these gametes must be relatively old and that they fail to conjugate for this reason. Finally, there are the particularly instructive cases in which old female gametes are beset by vigorous young males which have been but recently discharged. Although the males may swarm over the female gametes none succeed in fusing with them. Both gametes, apparently, have to be young and vigorous.

Instances of successful conjugations between old gametes (both at least fifteen minutes old) have been reported in an earlier paper (Hatch, 1933, p. 167), from which I quote:

'9.49 a.m. The last gamete in the female gametangium began pushing through the exit pore.' Since it usually takes at least fifteen minutes to empty a gametangium this gamete was probably more than fifteen minutes old.

'9.50 a.m. Before it could free itself a male moved up and apparently became attached by its anterior end.' This male while of unknown age could not in all probability be younger than the female, since the last male gametangium in dehiscence in this region was the subtending male and this had been discharged fifteen minutes.

'10.01 a.m. The two became separated but only momentarily, the male again applying itself to the female, this time with a vigorous lashing of its cilium, as if it were trying to force an entrance. This induced violent movements in the female, then both became quiet and so closely appressed that it was thought erroneously that fusion had been completed.

'10.15 a.m. The female began to blister and bubble over its whole surface. This was the start of the amoeboid movements that were to continue for ten minutes. Ciliary motion, however, did not cease. The associated cells now became much elongated. In this form they thrashed about, accompanying these motions with amoeboid movements that, on occasions, drew out the fusion cell into very odd shapes.

'10.25 a.m. The zygote became noticeably quiet and started sliding down over the surface of the gametangium towards the hypha.

'10.30 a.m. It rounded up, forty minutes after fusion began.'

Conjugations of the sort described above, however, are more spectacular than numerous, more bizarre than significant. More numerous and more typical are the quick and relatively unobtrusive fusions achieved by gametes less than fifteen minutes old. A case in point is that of the conjugation described below. It is as typical as any one instance of conjugation can be.

At 2.08 the female gametangium discharged its first gametes.

At 2.10 male gametes began to issue from the male gametangium.

At 2.13 in a survey of the female gametes three, already completely individualized, were singled out for closer observation. They were gathered in a little group, free from the others, and the only motion they showed was a gently swaying movement. They were also spherical, so that in neither motion nor shape did they show any reaction towards each other. Finally, they were densely granular as are all young and vigorous gametes.

At 2.14 a male gamete from the subtending male gametangium, in one of its erratic, zigzag dashes, swung into the centre of this group. No contact, however, was established between the male and any of the three female gametes unless it was achieved through their cilia. Nevertheless, a notable change came over both the male and the female gametes. The male immediately ceased his active ciliary movements and remained quietly in the midst of the females. The response of the females was an amoeboid movement in the direction of the male gamete. Slowly the female gametes closed in on the male.

At 2.17 after three minutes of this amoeboid activity the nearest female made contact with the male. They came together smoothly, slid about over each other momentarily, and then fused with a rush. Upon this abrupt mingling of cytoplasms a mad swirling and streaming ensued which resulted in bizarre distortion for the young zygote. It seemed to boil and bubble over its whole surface as its contents poured in and out of pseudopods which were retracted almost as soon as they were extended. While the zygote at this time scarcely lent itself to careful microscopical analysis, enough could be seen to determine that the cilia were, apparently, inserted at about the same spot on the zygote membrane, that the nuclei were drawn up side by side just below this point, and that the nuclear caps had fused. The question of how this alinement was achieved must be left unanswered temporarily.

At 2.24, after seven minutes of this ebullition, the zygote rounded up and swam away with a vigour and speed that made it very difficult, if not impossible, to follow it. Of the subsequent behaviour of the swarming zygote all that could be observed was that it tended to drop toward the substratum.

2. Observations on fixed material.

The act of conjugation between living gametes is so sudden as to prevent careful analysis. By fixation, however, this process can be halted so that conjugation may then be more effectively studied. Particularly significant in this regard is the fact that fixation apparently arrests conjugation instantly, even in its most violent phase. Those stages that can scarcely be detected in the living condition are consequently preserved.

It will be recalled that after contact was established between male and female gamete, they did not fuse immediately but slid over each other, their movements being barely perceptible or highly conspicuous, depending upon the age of the gametes. The significance of this is made clear in cytological preparations because they show that conjugation requires precise positional

adjustments between the gametes. Their membranes do not break down at any point on their surface but in a certain definitely prescribed region. This region is that which immediately surrounds the point of cilium insertion. When the ciliated ends are opposed, and not until then, gametes which are capable of fusion, fuse.

After this necessary orientation of the gametes is achieved there is probably, to judge from the momentary cessation of movement in living gametes, a short pause before the membranes actually collapse. From a study of living conjugants we know that with the collapse of the membranes the cells unite, but this throws no light on the actual process of conjugation. For this we must turn to cytological preparations.

Pl. XIX, Fig. 16, is of a male and female gamete about to establish contact. In these gametes the nuclei, nucleoli, and nuclear caps can be easily seen; the cilia can also be seen but much less distinctly. But even were the cilia indistinguishable, the ciliated end of the gametes could be quickly determined by noting the position of the nuclei and nucleoli for, as has been pointed out, the cilium of a gamete is connected with its nucleus by a rhizoplast, continuous through to the nucleolus by an intranuclear rhizoplast extension. In Pl. XX, Fig. 22, the two cells have come in contact, and the male has begun to crawl or roll over the female. The relative proportions of the nuclei, nucleoli, and nuclear caps in the two gametes are readily apparent. Quite as apparent is the fact that the ciliated ends of the two gametes have not yet been brought together. This essential adjustment is being made by the amoeboid movements of the male. In Pl. XIX, Fig. 17, and Pl. XX, Fig. 23 (the same conjugants), this adjustment has been achieved and the two cells are joined by a pore-like connexion. The collapse of the gamete membranes has occurred, and that collapse significantly enough has occurred at the bases of the cilia.

After this pore-like connexion is established the two cells proceed to 'fold' or 'swing' together. I say this advisedly, for while the roof of the pore, i.e. the membrane lying between the points of cilia insertion, suffers no significant distension the floor of the pore is depressed downward (Pl. XIX, Fig. 18, and Pl. XX, Fig. 24, same conjugants). As a broad connexion is established, probably by the expansive rush of cytoplasm, the nuclei and nuclear caps swing together. They do this, apparently, because the nuclei are suspended from the roof of the pore by their rhizoplasts, which significantly converge towards the gamete membrane where they connect up with their respective cilia. The points of cilia insertion being median to the nuclei and somewhat closer together than the nuclei, the latter must perforce swing together. As the nuclei move closer the nuclear caps are brought into contact. When they meet they coalesce (Pl. XIX, Fig. 20, and Pl. XX, Fig. 25). With the nuclei swung into place and the nuclear caps fused (Pl. XX, Fig. 26), conjugation is ostensibly complete. It only remains now for the zygote to show certain further accommodations. Foreign cytoplasm has to be mixed, unlike

lipoid granules have to be stirred together, and the nuclear caps have to amalgamate and adjust themselves to their new station. This is all accomplished, I suggest, in these wracking amoeboid movements that are so characteristic of post-conjugants. Shortly after, as we know, the zygote rounds up and swims away. Its appearance at this time is shown in Pl. XIX, Fig. 21, and Pl. XX, Fig. 27 (same conjugants).

By way of conclusion I should like to emphasize that conjugation in *Allomyces arbuscula* is essentially a 'folding together' of male and female gamete. The male does not empty itself into the female or the female into the male, but both empty towards the point of rupture, a common, neutral, median ground, and there reorganize the new cell, the zygote. It is true, of course, that in the first rush of cytoplasm one conjugant may momentarily receive a disproportionate amount. See Pl. XIX, Fig. 19, and Pl. XX, Fig. 25, where the male has a greater volume than the female. This is only a temporary condition, however, and is quickly adjusted as the zygote organizes itself around the centrally disposed nuclei.

Before leaving the subject of conjugation I should also like to call attention to the fact that while the motion of the gametes prior to conjugation is relatively restrained, that of the zygote, the product of conjugation, is very vigorous. Its amoeboid activity is violent and when it begins to swarm it swims with great rapidity, dashing about most energetically, more so, perhaps, than the male gametes at the height of their activity. It is as though the zygote gained considerable vigour as a result of conjugation.

With the formation of the zygote conjugation might be considered at an end, but, although two cells have been combined, and two cytoplasms have been mixed, the male and female nuclei have yet to fuse. To locate the time of nuclear fusion and to trace the progress and the process of this fusion it is necessary to consider the history of the zygote. As we have noted, the first few minutes of a zygote's existence are given over to violent ebullition. It then rounds up and swims away. While it swims with great vigour it nevertheless gradually settles in the water and thus, sooner or later, brings up on the substratum. Here it becomes amoeboid. After some minutes of amoeboid activity it rounds up and movement permanently ceases. In this quiescent state the cilia are cast off and the nuclear cap breaks down, becoming completely dispersed through the cytoplasm. After swelling slightly the zygote germinates by rhizoidal germ-tubes. The first of these developments to affect the nuclei in any significant way is the casting of the cilia. In the sloughing of the cilia the rhizoplasts connecting the cilia and the nuclei are destroyed. Since it was the rhizoplasts that drew the nuclei close to the zygote membrane, their collapse causes the nuclei to be set loose (Pl. XXII, Fig. 37). But the important development, as far as nuclear fusion is concerned, is the dissociation of the nuclear cap. The progressive disintegration of this body is shown in a series of photomicrographs (Pl. XXI, Figs. 28-33). The zygote of Fig. 28 has but recently come to rest, and but recently lost its cilia.

Its fused nuclear caps are well concentrated and stain heavily. Its peripheral cytoplasm is relatively clear. The zygotes of Figs. 29 and 30 illustrate two stages in the dispersion of the cap substance. In Fig. 31, although the dispersion is almost complete, two small masses yet remain to be dissipated. In Fig. 32 it is complete, and in Fig. 33 germination has begun. This same series is illustrated in Pl. XXII, Figs. 40-5. The relationship between the dissociation of the nuclear cap and nuclear fusion is that only with the dissociation of the nuclear cap do the nuclei become free to fuse.

Prior to the dissociation of the nuclear cap the male and female nuclei often become widely separated in the cellular displacements experienced by the amoeboid zygote. Sometimes, however, the nuclei retain their side-by-side position (Pl. XX, Fig. 27). The degree of association between male and female nuclei prior to nuclear cap dissolution is, then, no measure of progress towards an eventual nuclear fusion. Only after the nuclear cap begins to break up and the nuclei are 'freed', so to speak, is the association between male and female nucleus any indication of the imminence of nuclear fusion. Thus, in Pl. XXII, Fig. 40, the close association of male and female nucleus probably means very little, whereas that of the nuclei in Pl. XXII, Fig. 42, is probably of some significance. The justice of this assumption is borne out by the fact that since nuclear fusion is often accomplished before the cap substance has been wholly dispersed (Pl. XXII, Fig. 43), effectual nuclear contact must often be established at a stage in nuclear cap dispersion comparable to that exhibited in Pl. XXII, Fig. 42.

The manner in which nuclear fusion is achieved is shown in Pl. XXI, Figs. 30-3, and in Pl. XXII, Figs. 42-5. In Fig. 30 the nuclei and nucleoli lie in the same focus. An opportunity is thus offered to make a comparative study of male and female nuclei, male and female nucleoli. In this and other zygotes, where the male and female nuclei are actually in contact, the nucleoli very often appear to be displaced towards the point of contact. It is far from certain, however, that there is any real significance in this opposition of male and female nucleoli, since fusion can apparently be accomplished by nuclei whose nucleoli are not so precisely orientated. The best explanation of the frequent occurrence of this opposed orientation is to be found, I believe, in the nuclear alignments achieved at conjugation where the nuclei are brought in contact by their nucleolated ends. When this alignment is not disturbed the nucleoli would naturally be left facing each other across their adjoined membranes. Pl. XXI, Fig. 31, and Pl. XXII, Fig. 43, illustrate instances of actual nuclear fusion, Pl. XXI, Fig. 32, and Pl. XXII, Fig. 44, the appearance of fusion nuclei. These particular nuclei are interesting because the male and female nucleoli have not yet fused as they shortly will, and as those of Pl. XXI, Fig. 33, and Pl. XXII, Fig. 45, actually have. The nucleus of Pl. XXII, Fig. 44, illustrates still another point in that the chromatic reticulum abutting on the female nucleolus is clearly looser than that drawn about the male nucleolus.

From the foregoing account of the nuclear developments in the zygote it is apparent that in point of time nuclear fusion is more closely associated with the germination of the zygote than with the act of conjugation. This is not always the case, for very occasionally planozygotes are found in which nuclear fusion has already been achieved. Why should the nuclei in an occasional zygote fuse quickly, yet those of an overwhelming proportion fail to fuse until the time of germination? The explanation lies, I believe, in the fact that during conjugation the intrusion of nuclear cap material between the nuclei may be great, or little, or none at all. In view of the fact that a wall of intact cap substance apparently acts as a barrier to nuclear fusion it seems entirely logical to conclude that when there is an intrusion of cap substance nuclear fusion is delayed until zygote germination; but that when there is no intrusion nuclei fuse quickly, possibly at the time of conjugation or shortly thereafter (Pl. XXII, Fig. 47). In analysing this situation it is interesting to note that there is no intermediate condition: the nuclei fuse either early, at conjugation, or late, at germination. Of the overwhelming number of nuclei that fuse at the time of zygote germination it is safe to say that no more than one-half fuse prior to germination (Pl. XXI, Fig. 32, and Pl. XXII, Fig. 44), the rest fusing after germination (Pl. XXI, Fig. 36). In some instances germination may be far advanced before the male and female nuclei finally fuse. The zygote in Pl. XXI, Fig. 36, is a case in point.

V. ZYGOTE GERMINATION

When a cell specialized for a free-swimming or an amoeboid existence settles down, throws out germ-tubes, and begins to develop a mycelium, it is apparent that some very extraordinary adjustments have been made by that cell. The more spectacular changes include the casting of cilia and the extension of germ-tubes, but the fundamental change, of which this is possibly only an expression, is the profound change that must take place in the protoplasm of the cell. In the germination of most zygotes, or zoospores for that matter, this fundamental adjustment is accomplished so unobtrusively that our knowledge of their germination is still very superficial. With the zygote of *Allomyces arbuscula* germination is intimately associated with the dissociation of a large nuclear cap, a cytoplasmic inclusion that can be easily seen and easily followed through the process of germination.

Since it is impossible to state *a priori* just what renders a zygote fit for germination or when these conditioning changes occur, we may begin our analysis with the planozygote.

The planozygote (Pl. XIX, Fig. 20, and Pl. XX, Fig. 27) is biciliate with both cilia inserted at approximately the same point on the zygote membrane and, unless it be one of those very occasional zygotes in which the nuclei fuse at conjugation, it is binucleate. Since the cilia are connected with the nuclei by rhizoplasts, the two nuclei are drawn close to the zygote membrane just opposite the point at which the cilia are inserted. A common nuclear cap

invests these nuclei so closely that a wedge of cap substance is thrust between the nuclei. The cap at this time has a sharp, well-defined edge and stains heavily.

While the planozygote may take a few short swarming flights after it first falls to the substratum, it more often ceases its ciliary activity immediately and starts exploring its new environment in halting amoeboid movements. These movements, which soon become very vigorous, result in curious internal transformations. The nuclear cap, for instance, is conspicuously affected. In Pl. XXII, Fig. 38, the nuclear cap has simply been folded back on itself. In Pl. XXII, Fig. 37, however, a small blob is in the process of being drawn into a pseudopod. In Pl. XXII, Fig. 39, most of the cap has been displaced from about the nuclei, and continued amoeboid movements threaten to disperse and fragment it further by pinching off one or both of the two arms into which the bulk of the cap has been concentrated. Finally, in Pl. XXII, Fig. 47, where the main body of the cap has been displaced from about the nuclei, another effect of amoeboid activity can be seen. This type of displacement is particularly common. The question of the varied disposition of the cap need give us no further concern, however, because by the time the zygote germinates and the substance of the cap enters significantly into the metabolism of the cell that substance will have become evenly dispersed through the cell.

In the amoeboid phase one of the rhizoplasts may occasionally be broken. Before the zygote illustrated in Pl. XXII, Fig. 38, became amoeboid it was organized and orientated like the planozygote of Pl. XX, Fig. 27. In the attainment of the peculiar amoeboid condition it now exhibits, two pseudopods must have been organized towards what was then the ciliated end of the gamete, the upper end as we view it. Of these two pseudopods one apparently drew away to the left and arched up over the zygote. The other swung off to the right and beneath the zygote. The male nucleus was caught up in the first, the female in the second, and both were carried to the position shown in Pl. XXII, Fig. 38. For the female nucleus this displacement meant the loss of its cilium, which structure was borne off on the tip of the other pseudopod. The consequence to both nucleus and cilium, when such a break occurs in the rhizoplast, is shown in Pl. XXII, Fig. 39. With the rhizoplast connexion broken, the female nucleus is no longer held in any fixed position in the cell, but becomes free in the cytoplasm where it is tumbled about in subsequent amoeboid movements. The cilium is thrown off. Whether this means that the cilium is as dependent on the nucleus for anchorage as the nucleus is dependent on the cilium is not easily answered. It would, however, seem to be indicated.

In the remarkable instance of amoeboid activity described above (Pl. XXII, Fig. 38), while it would appear that an amoeboid process had literally ripped the female cilium from the female nucleus, there is really no means of proving that the nucleus had not disengaged itself from its cilium before the pseudopod bore it away. There is, then, some doubt even in this case, whether amoeboid movements can accomplish cilia-casting unaided. Since no instance has ever

been seen where amoeboid activity could be held solely accountable for the sloughing of cilia, the probability is that amoeboid movements are only incidental to another and a more fundamental process, the nature of which should become clearer as we proceed with the analysis of what occurs when the zygote comes to rest (Pl. XXI, Figs. 28–36, and Pl. XXII Figs. 40–5).

In zygotes that have but recently abandoned amoeboid activity, the cilia, both of them, are conspicuously absent (Pl. XXI, Fig. 28, and Pl. XXII, Fig. 40). That they were sloughed off, not reabsorbed, is suggested by the fact that in zygotes where one cilium has been lost (Pl. XXII, Fig. 39), the other cilium is still whole and shows no evidence of even partial resorption. If resorption was the method employed in removing the first cilium it might be confidently expected that the second cilium would be at least partially resorbed. Supporting evidence is to be gathered from those zygotes where whole cilia have broken away from their nuclei (Pl. XXII, Fig. 38).

In cilia divestment the connexion between the nucleus and the cilium is always broken. This is indicated in Pl. XXII, Fig. 39, and it is proved by the fact that in all zygotes lacking cilia (Pl. XXII, Figs. 40–2) the nuclei fall away from the membrane of the zygote. In all ciliated zygotes the rhizoplast connexions between nuclei and cilia hold the nuclei close to the membrane (Pl. XX, Fig. 27, and Pl. XXII, Figs. 39, 46, and 47). If cilia are sloughed off and this casting is anticipated by a break in the rhizoplasts, as seems reasonably certain, we now have only to determine where the changes arise that weakened the rhizoplasts. Do they arise from some internal change within the rhizoplasts themselves or from some changes external to the rhizoplasts, i.e. in the cytoplasm through which the rhizoplasts pass? While it is true that the cilia, hence the rhizoplasts, become useless when the zygote comes to rest, and while it might be argued that loss of function might initiate changes within the structure that might conceivably result in the loss of that structure, this line of argument is more or less vitiated by the fact that the loss of ciliary function in gamete or zygote apparently does not, *per se*, result in the loss of cilia. This is most clearly indicated in the behaviour of gametes, for while they regularly continue to live for some time after they have ceased effective ciliary activity, barring parthenogenetic development, they do not discard their cilia. Likewise, when the zygote exchanges ciliary activity for amoeboid movement it does not cast its cilia immediately but carries them through all its amoeboid activity, and discards them only when it rounds up and settles down. Since loss of function by cilia apparently has little effect on the casting of the cilia it seems distinctly improbable that changes originating within the motor apparatus itself sufficiently account for the dissolution of the rhizoplasts. The weakening of the rhizoplast would seem to be effected from without, i.e. from changes in the cytoplasm in which the rhizoplasts lie. Such changes are conspicuous, involving as they do the dissociation of the bulkiest inclusion in the cell, the nuclear cap.

While it is very difficult to determine just when the dissociation of the

nuclear cap starts, it seems entirely probable that it may begin before the zygote settles down; if later, then at some time prior to the sloughing of the cilia. The reasons for carrying the beginning of nuclear cap dissociation back into the amoeboid zygote, or at least to a period antedating cilia divestment, are two. One is that the gross dissociation of the cap substance becomes evident too quickly after the cilia are cast to have been initiated subsequent to that development. Changes such as this usually start slowly. The second point is that cilia divestment is apparently brought on by changes in the cytoplasm. The only cytologically demonstrable change occurring in the cytoplasm being the dissociation of the nuclear cap, it would appear as though the dissociation of the cap might in some way effect the sloughing of cilia. To do this, however, it must, at least in its beginning, precede the sloughing of the cilia.

After cilia divestment the course of nuclear cap disintegration can be easily followed, and since its progressive dissociation can be most accurately and most effectively followed in a series of photomicrographs a plate has been prepared illustrating this (Pl. XXI, Figs. 28-33). The zygote of Fig. 28 has but recently come to rest, has but recently lost its cilia. Its fused nuclear caps are well concentrated and stain heavily. Its peripheral cytoplasm is relatively clear. The zygotes of Pl. XXI, Figs. 29 and 30, illustrate two stages in the dispersion of the cap substance. In Pl. XXI, Fig. 31, although the dispersion is almost complete, two small masses yet remain to be dissipated. In Pl. XXI, Fig. 32, it is complete, and in Pl. XXI, Fig. 33, germination has begun. A series of drawings has been made to supplement and to help in the interpretation of these photomicrographs (Pl. XXII, Figs. 40-5).

Before claiming for the dissolution of the nuclear cap any special and exclusive significance in conditioning the zygote for germination, the role of the nuclei should at least be examined. It is apparent that in some zygotes nuclear fusion occurs prior to germination (Pl. XXI, Figs. 31 and 32, Pl. XXII, Figs. 43, 44), but that in others, representing nearly one-half of all the zygotes, germination begins, may even be far advanced, before nuclear fusion is achieved. The zygote in Pl. XXI, Fig. 36, is a case in point. To show the male and female nuclei, only the tip of the germ-tube could be brought into focus. It can be seen between the two gametes in the lower left-hand corner. Germination, then, runs its course irrespective of the nuclei. With the dissociation of the nuclear cap it is very different, for germination never occurs in its absence and is never long delayed after it is complete. The disintegration of the nuclear cap thus appears to be the fundamental protoplasmic adjustment that conditions the zygote for germination. That it has an effect on the cytoplasm is fairly certain, for it seems incredible that such a striking metamorphosis as complete disintegration could occur in the largest cytoplasmic inclusion without affecting the rest of the cytoplasm. For a demonstrable effect the dissolution of the rhizoplasts can be mentioned, for, as we have seen, the disintegration of the nuclear cap apparently induces

a physiological rejuvenescence in the cytoplasm capable of accomplishing this. A partial answer as to how it is accomplished is to be found in the fact that during the time that the nuclear cap is breaking up and for some time prior to actual germination the zygote increases in volume. Since a general increase in volume precedes germination the first effect, the primary and fundamental effect, of nuclear cap dispersion is, apparently, growth. Germination is but a secondary effect, is but another expression of growth, a special and a localized type of growth. Thus it would seem that the dissolution of the nuclear cap initiates germination by causing that cell to grow.

The question arises as to how the dissolution of the nuclear cap causes a cell to grow? A very plausible explanation lies in the fact that chondriosomes are returned to cytoplasmic circulation when the nuclear cap breaks up. Now chondriosomes and growth are intimately associated in *Allomyces arbuscula*. In actively growing hyphal tips, for instance, the chondriosomes are numerous and their concentration is densest where the cellular metabolism is highest, namely in the tip. In the bulging of the gametangia during the early stages of gametogenesis and in the extension of the germ-tubes of zygotes at germination, wherever there is growth, chondriosomes are abundant and, conversely, wherever there is a notable concentration of chondriosomes there one finds growth.

The relationship between chondriosomes and growth is shown to advantage in the germination of the zygote, for starting with a cell in which there are no chondriosomes one can watch the effect on the cell of the return of chondriosomes to cytoplasmic circulation. As the cap is breaking up, freeing progressively more and more chondriosomes, the zygote swells. There is, then, this morpho-physiological effect. The cytological picture changes, too, as a comparison of a planozygote and a germinating zygote will show (Pl. XIX, Fig. 21, Pl. XXI, Fig. 33, Pl. XX, Fig. 27, and Pl. XXII, Fig. 45). The cytoplasm, once so clear, becomes darker and more granular as the chondriosomes move into the cytoplasm. In that initial phase, when the chondriosomes are evenly distributed through the cytoplasm, growth is general; the whole cell swells. When the chondriosomes concentrate in the basal end of the zygote, as they shortly do, the extension of a germ-tube is imminent. Thus a localization of chondriosomes results in localized growth.

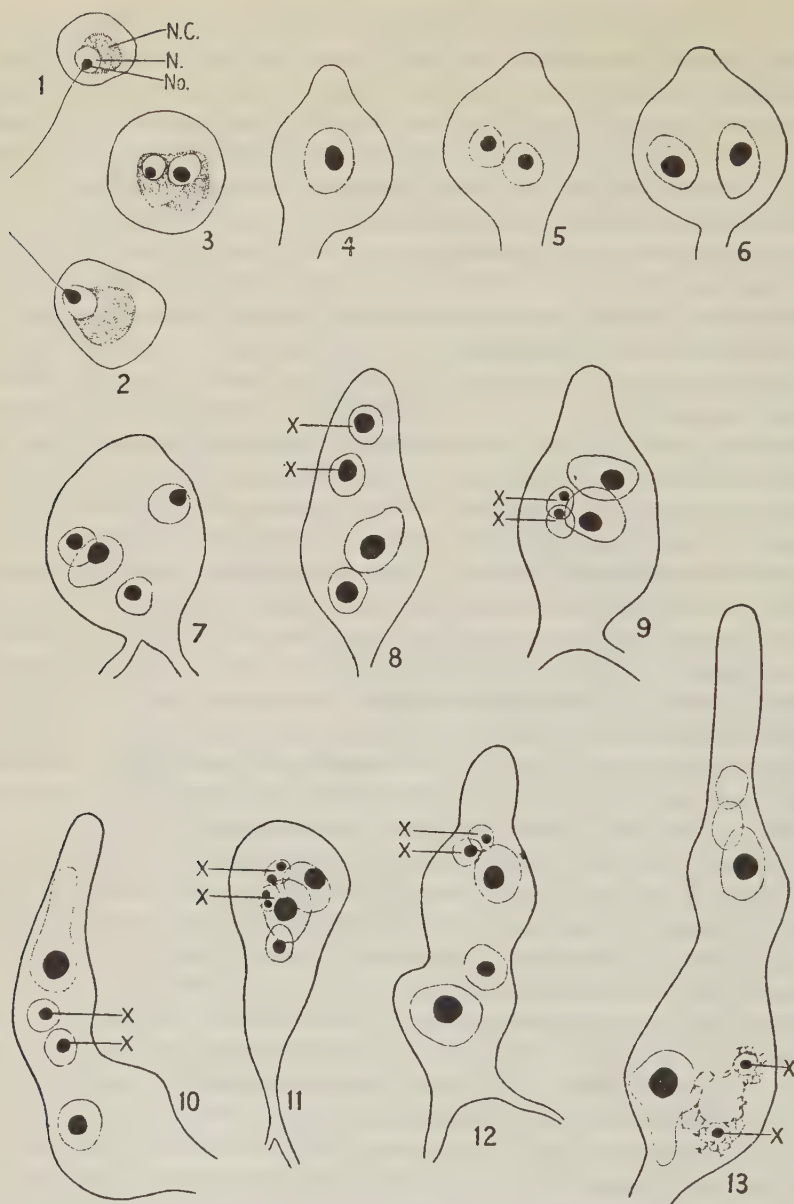
The initial germ-tube to appear on the zygote is at first of small diameter and of approximately the same width throughout (Pl. XXI, Fig. 33, and Pl. XXII, Fig. 45). Very shortly, however, it begins to thicken proximally so that it develops a distinct taper (Pl. XXI, Fig. 35). When this germ-tube first appears a knot of chondriosomes crowds into it. As it grows, a weft of chondriosomes is carried along in its advancing tip and out into the tips of branches when these develop. Lipoid granules also move out into the rhizoidal system, but nuclei never leave the body of the zygote, for their diameter is too great to permit them to push into the base of the rhizoid. Since the rhizoidal germ-tube grows very rapidly and branches profusely an extensive,

tapering rhizoidal system is soon laid down. When, and only when, this has become extensive does the body of the zygote begin to bulge apically (i.e. at the end opposite the rhizoids). In this bulge the chondriosomes are again prominent. They crowd into it, and while a few lipoid granules may wander in behind them the chondriosomes are by far in the majority. This bulge, which is the beginning of the hyphal germ-tube, is relatively broad, so much broader than the rhizoidal germ-tube that the nuclei can move into it easily.

An immediate and a very interesting result of the growth of the zygote, both before and after germination, is to be found in the size of the nuclei. As the cytoplasmic volume increases the nuclear volume increases in the same ratio. In male and female gametes a certain ratio is established between nuclear and cytoplasmic volume (Pl. XVIII, Figs. 1 and 2, Text-figs. 1 and 2). This ratio, as nearly as can be computed, is the same for both cells, the smaller male nucleus maintaining the same balance with its smaller volume of cytoplasm as the larger female nucleus does with its larger volume of cytoplasm. Conjugation, since it combines two balanced systems, simply perpetuates this balance. In the planozygote and in any zygote up to the time of its pre-germination enlargement, there being no increase in the amount of cytoplasm, no change is seen in the total nuclear volume (Text-fig. 3). Thus, from the time the gametes are formed until the zygote prepares to germinate, nucleus and cytoplasm preserve a static equilibrium. From the time of germination onwards, however, nuclei and cytoplasm are in dynamic equilibrium, for the cytoplasm is increasing in amount, the nuclei increasing in size. To analyse this it is best to consider only the earliest stage of germination, that stage which comes to an end when the fusion nucleus divides. This suggestion is made because meiotic phenomena complicate the picture thereafter.

In those zygotes where germination begins before the male and female nuclei have fused, both nuclei enlarge and both enlarge proportionately. When the gamete nuclei fuse before germination the fusion nucleus makes this adjustment (Text-fig. 4). Considering that the nucleus of the female gamete is but $3.6\ \mu$ in diameter and that of the male only $2.3\ \mu$, the fusion nucleus, with a diameter of at least $6\ \mu$, has a volume considerably greater than would be expected. This increase in volume over that of the combined male and female nuclei is apparently an adjustment of the nucleus to the effect of germination upon the nuclear-cytoplasmic ratio.

The existence of a dynamic nuclear-cytoplasmic ratio does not end with the fusion nucleus, for it affects the daughter nuclei quite as strikingly as it does the gamete and fusion nuclei. In Text-figs. 5 and 6, for example, the daughter nuclei in the larger and older zygote are approximately twice the size of those in the smaller and younger zygote. In still older germlings, four-, five-, and six-nucleate zygotes (Text-figs. 7-13) the picture is complicated by the fact that two of the nuclei (marked with an x) are degenerating from causes quite apart from those inherent in a nucleus-plasma ratio. The other nuclei are normal enough and their size is apparently an expression of this ratio. They



TEXT-FIGS. 1-13. A series of outline drawings illustrating nuclear size and nuclear degeneration in the germinating zygote. $\times 1,750$. Fig. 1. Male gamete. Fig. 2. Female gamete. Fig. 3. Ungerminated zygote in which the male and female nuclei have not yet fused. Figs. 4-13. Germinated zygotes. Fig. 4. Fusion nucleus just prior to its division. Fig. 5. A binucleate zygote. Fig. 6. A larger and older binucleate zygote showing in the enlargement of its nuclei the effect of cellular growth on nuclear size. Figs. 7 and 8. Four-nucleate zygotes in which the nuclei are of about the same size. Figs. 9 and 10. Four-nucleate zygotes in which two of the nuclei have clearly lost volume. Figs. 11 and 12. Five-nucleate zygotes in which the

are, however, very different in size. This means, for one thing, that in the germinating zygote of *Allomyces arbuscula*, nuclear size has apparently no genetic significance. The nucleus in Text-fig. 4 is the fusion nucleus and has the $2n$ chromosome number. The nuclei in Text-figs. 5-13 have but the n chromosome number and yet some of them are as large as, some larger than, the $2n$ fusion nucleus. Nor is there agreement in size between nuclei of the same chromosomal constitution. The two larger nuclei of Text-fig. 10, for example, both have the haploid number of chromosomes and yet one is much larger than the other. The three larger nuclei of Text-fig. 11 show the same lack of agreement, the largest being three, possibly four, times as large as the smallest. In Text-figs. 12 and 13 much the same sort of disagreement exists in the size of the nuclei. But can a nuclear-cytoplasmic ratio explain this variation in nuclear size? It can do so, if we postulate that local concentrations of cytoplasm, working within the ratio, control the size of the nuclei in their vicinity; hence the presence of larger and smaller nuclei. Since the internal constitution of the nuclei apparently has nothing to do with it, some external factor must be sought, and what better than this ratio?

This discussion of nuclear size in the zygote explains why so little mention is made of nuclear size in the subsequent description. Except as an expression of the nuclear-cytoplasmic ratio it seems to be of no significance. Now, the zygote is not an isolated system, for elsewhere in the ontogeny of the thallus the same conditions obtain, though they may not be so extreme. Thus, if one would read genetical implications into nuclear size the operation of the nuclear-cytoplasmic ratio should always be taken into account. Care should also be taken to discover the mean of a great many nuclei. To do this whole thalli should be studied.

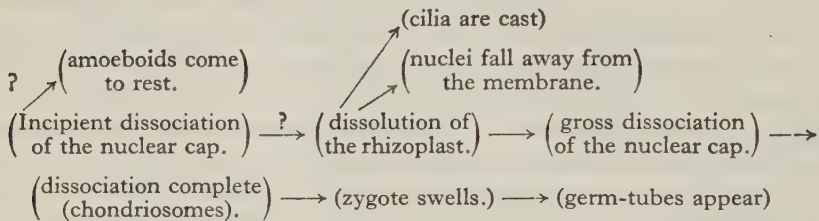
In analysing the whole series of adjustments made by a zygote from the time it ceases its motility until it extends its first germ-tubes, it seems justifiable to interpret germination as a sort of chain reaction which is set in motion by the dissolution of the nuclear cap. According to this interpretation germination may actually begin while the zygote is still motile, i.e. in the amoeboid zygote, or if a little later, then some time in the early history of the stationary zygote. The argument is as follows: cilia do not drop off the zygote and nuclei do not change their position without cause, but this, as we have seen, is brought about by the collapse of the rhizoplasts. As to the cause of the rhizoplast collapse, everything points to a subtle change in the cytoplasm through which these rhizoplasts pass (pp. 600 and 601) and the only thing likely

diminution of the two smaller nuclei has progressed still further. Note that the retrograding nuclei in Fig. 11 are both binucleate and that among the three larger nuclei there is a very considerable variation in size. Fig. 13. A six-nucleate zygote in which degeneration has overtaken the retrograding nuclei. Their nuclear membranes have broken down and their chromatic reticulums have become so dispersed that their nucleoli are now all that identifies them. N. = nucleus; N.C. = nuclear cap; NO. = nucleolus; x. = degenerating nuclei.

Magnifications stated in these 'Explanations' are based on the requisite reductions in all cases.

to effect this change is an incipient dissociation of the nuclear cap. That the dissociation of the nuclear cap does have an effect on the cytoplasm is certain. That the initial effect should be the dissolution of the rhizoplasts seems likely. Thus the circumstances behind the casting off of cilia point to a prior change in the nuclear cap. The dissociation of the nuclear cap, accordingly, becomes the key reaction that starts the whole process and marks the beginning of germination. How much it antedates cilia divestment is not known, but it is entirely possible that it is incipient dissolution of the nuclear cap that causes the zygote to come to rest in the first place. After the zygote has lost its cilia and after the nuclei have taken a central position in the cell, gross dissociation is exhibited by the nuclear cap and it breaks down completely. During this process and for a short time thereafter the zygote swells. Finally, after these internal adjustments have been made, the germ-tubes appear. Germination in *Allomyces* accordingly implies considerably more than the simple extension of germ-tubes.

THE PROCESS OF GERMINATION



While the actual existence of any such chain reaction is largely hypothetical there is good reason to believe that cilia divestment is definitely associated with the process of germination. Negative evidence, i.e. the fact that loss of cilia cannot apparently be correlated with loss of ciliary function, suggests that germination might effect divestment. Positive evidence of a correlation between cilia divestment and germination is suggested by the germination of parthenogenetic gametes, for these gametes regularly discard their cilia. Since no normal gamete ever casts its cilium, and since the only activity experienced by the parthenogenetic gametes that is not shared by the normal gametes is germination, it would appear that it is germination alone that effects ciliary divestment. While this relationship between germination and the sloughing of the cilia cannot be demonstrated so sharply in the case of the zygote, because there are no non-germinating controls, it does apparently exist, for the actual casting of cilia is regularly postponed until the amoeboid zygote has rounded up and germination is imminent.

After zygote germination there is nothing further to record of the nuclear cap, the structure which appeared in the gametes at gametogenesis as the result of a concerted aggregation and coalescence of chondriosomes, that underwent no detectable change during the swarming of the gametes, that

played an entirely passive role during conjugation, that was distorted by, but apparently initiated none of, the displacements effected in the plano- or amoeboid zygote. In the gamete, and in the zygote up to the time it settles down, the nuclear cap apparently has no function. From the minute, however, that the nuclear cap begins to break up it apparently dominates both the physiological and morphological activities of the cell: physiological, because the return of the chondriosomes to cytoplasmic circulation, implied in their break-up, gives every evidence of initiating a cytoplasmic rejuvenescence; morphological, because it results in the formation of germ-tubes, ultimately, a mycelium.

VI. ZYGOTE GERMINATION AND MEIOSIS

At germination the zygote experiences two critical nuclear divisions which are of importance in a correct interpretation of the life-cycle of this fungus. Is this the point at which chromosomal reduction occurs? To investigate this matter a technique had to be devised (p. 585) whereby a great many zygotes could be obtained in the proper stage of development, i.e. at the stage when the body of the zygote first begins to bulge apically in anticipation of the extension of the hyphal germ-tube.

Our first concern in this study is with the chromosome number of the fusion nucleus. This, fortunately, is already known, for in an earlier study (Hatch, 1935) it was discovered that the chromosome number of gamete nuclei is six. With each gamete contributing six the chromosome number in the fusion nucleus of the zygote must be twelve. Since chromosome counts can most easily and convincingly be made at metaphase, a careful analysis of a series of metaphase figures has been made.

In the two nuclear divisions associated with zygote germination the orientation of the spindle seems to be a casual matter, for it may lie parallel, oblique, or perpendicular to the long axis of the zygote. The spindle is intranuclear (Pl. XXII, Figs. 49-53). Outside this spindle, but still within the nuclear membrane, there is a nucleolus—sometimes there are two. In definitely placing the nucleolus outside the spindle I am not unmindful of the fact that in Pl. XXII, Fig. 48, an oblique polar view, the nucleolus appears to lie in the centre of the metaphase plate. This is, I believe, an optical illusion, since the nucleolus actually lies on the far side of the spindle; it appears within the spindle simply because the spindle has so little depth. In all the other zygotes figured (Pl. XXII, Figs. 49-53) and all other zygotes studied, the nucleolus clearly lies outside the spindle. Quite occasionally the nucleolus or nucleoli are set in little pouches in the nuclear membrane. The nucleolus persists through the metaphase and on into the anaphase.

The chromosomes themselves are remarkable structures, for despite their size (the largest and longest cannot be over $1\ \mu$ in length) they show a distinct individuality (Pl. XXII, Figs. 48-50, 52, and 53). The haploid number is six. Of these one is small, one is of medium size, and four are of a larger size.

But even among these four chromosomes, differences in size and possibly in shape can be seen. The fact that these individual differences are visible not only in this first division but also in the next and in all subsequent divisions inspires confidence in the chromosome counts.

With this background it is now possible to consider the disposition of the chromosomes in the important first division of the fusion nucleus. On the metaphase plate the twelve chromosomes become arranged in six pairs, which pairs appear to be homologous because the small chromosome pairs with the small chromosome, the medium sized with the medium sized, and the large chromosomes with the large chromosomes. This arrangement can be made out very clearly in a polar view of early metaphase chromosomes (Pl. XXII, Fig. 48, an oblique polar view). During metaphase the two homologous chromosomes in each of the six pairs separate and move towards opposite poles—six to a pole (Pl. XXII, Fig. 49, a side view of the metaphase plate). A reduction in chromosome number is obviously accomplished in this division. We can accordingly consider this the first meiotic division.

Characteristic features of the second meiotic division are that it follows quickly after the first, that the nuclei often divide simultaneously (Pl. XXII, Figs. 50–2), and that the chromosomes, i.e. the post-metaphase chromosomes, are clearly smaller than the chromosomes at a like stage in the first division (Pl. XXII, Figs. 50, 52, and 53, vs. 49).

The fact that the second meiotic division follows quickly upon the first is attested by the fact that the four-nucleate condition is almost always attained before the hyphal germ-tube has made any appreciable growth (Text-figs. 7 and 8). The zygote in Pl. XXII, Fig. 53, is an extreme case, for it is very rare indeed for the hyphal germ-tube to reach the proportions it has achieved in this zygote before the meiotic divisions have been completed. The fact that these second nuclear divisions should follow so quickly upon the first is rather remarkable, for after the first division there has been no great increase in the volume of the cytoplasm, only in that of the nuclei, and this condition would be against further nuclear division if division were simply a response to an unfavourable nuclear-cytoplasmic balance. Something else must effect it, and a meiotic mechanism involving two quick divisions meets the requirement very satisfactorily.

In Pl. XXII, Figs. 50–2, the division of the two daughter nuclei has been simultaneous. In Pl. XXII, Fig. 50, both nuclei could be drawn. In Pl. XXII, Fig. 51, neither could be drawn well because one was superimposed on the other. In Pl. XXII, Fig. 52, only one was drawn. This second meiotic division, however, may not always be simultaneous (Pl. XXII, Fig. 53). The division of the second nucleus, however, is never long delayed, for the four-nucleate stage is always achieved before the hyphal germ-tube has developed much beyond the stage noted in Pl. XXII, Fig. 53. The fact that the second meiotic divisions should either be simultaneous or follow so quickly on the other may not seem remarkable or even unusual. It is, however, only here in the zygote that

anything like a simultaneous division of all the nuclei in a cell can be really said to occur. In the hyphae, in resting sporangia, and in the gametangia, two, three, or even four nuclei may be in division at the same time, but in these cases there may be thirty, forty, or even seventy other nuclei in the segment or cell that are not dividing.

The size of the chromosomes in the second meiotic division is a noteworthy feature of that division. If the chromosomes in Pl. XXII, Figs. 50, 52, and 53, particularly those in Pl. XXII, Figs. 52 and 53, are compared with those in Pl. XXII, Fig. 49, it is apparent that the chromosomes of the second are smaller than those of the first division. The discrepancy in size is very clear in the material itself, but in drawing objects as small as these chromosomes it is difficult to maintain the proper proportions. Hence the distinction, while apparent, is not as clear in the drawings as in reality. The significance of this difference in size, if we are justified in attaching any significance to it, is that the anaphase chromosomes of the second division are monads having but a single chromatid, while those of the first division are dyads each bearing two chromatids.

To complete the study of meiosis in *Allomyces arbuscula*, the fate of the four nuclei produced by these two divisions has to be considered. By following the course of zygote germination through to the six-nucleate stage it can be demonstrated that two of these nuclei degenerate. In the four-nucleate zygote of Text-fig. 7 the nuclei are of approximately the same size. In the next zygote, slightly more advanced in germination (Text-fig. 8), there are also four nuclei, but two of these, namely those in the base of the hyphal germtube, are somewhat smaller than the two in the base of the zygote. In older zygotes (Text-figs. 9 and 10) two of the four nuclei in each case are clearly smaller than the other two. These smaller nuclei, to judge from the size of the two smaller nuclei in Text-fig. 8, have apparently suffered a slight but appreciable loss in volume. In five-nucleate zygotes (Text-figs. 11 and 12) a further diminution in size can be seen in the pair of small nuclei. That this progressive diminution is but incipient degeneration is apparent when still older zygotes are studied, for in them the collapse of the nuclear membranes occurs and the final elimination of these nuclei is brought about. A case in point is shown in Text-fig. 13, a six-nucleate zygote. The nuclear membranes have broken down, the chromatin is dispersed, and were it not for the nucleoli, the fate of these nuclei would have been difficult to determine.

That two of the four original nuclei degenerate is interesting, but what makes it significant is the fact that these two nuclei are apparently sister nuclei. The evidence for this is threefold.

1. The position of the degenerating nuclei. If in the four-nucleate stage it could be shown that the two nuclei destined to degenerate were always found in the base of the zygote or in the tip or in any fixed position, it would be permissible to conclude that the difference between the two nuclei that degenerate and the two that survive was the position they held in the zygote.

That this is not the case is clear from a study of the four-nucleate zygotes (Text-figs. 7-10), for here the degenerating nuclei may as often be found in the dense cytoplasm towards the tip as in the vacuolate cytoplasm toward the base or in any intermediate region. If the difference between the two pairs of nuclei does not seem to be due to their position in the zygote, which implies differences in concentration and possibly differences in the quality of the cytoplasm, the logical deduction is that the difference between the two pairs of nuclei must lie in the nuclei themselves.

Considering next the intimate association of the two degenerating nuclei, another consideration suggests itself, namely, that their association may not be casual but may actually be related to the fact that they have always been intimately associated, as sister nuclei.

2. The size of the degenerating nuclei. Beginning with the zygote of Text-fig. 8 and following through the series to Text-fig. 12, the areas of the paired degenerating nuclei were measured with a planimeter from camera lucida drawings. In this study a great many more zygotes were drawn and a great many more degenerating nuclei were measured than are represented in these figures. The results of these measurements showed that in their cross-sectional areas these paired nuclei were practically identical. This also suggests that these nuclei have a common inheritance.

3. The binucleolate condition of the degenerating nuclei in Text-fig. 11. This, I think, is the most conclusive evidence that these are sister nuclei. The other three nuclei in this zygote have but a single nucleolus.

Considered separately, none of these characteristics of the degenerating nuclei, either their intimate association, or their similar size, or their occasional binucleolate condition could be considered as anything like conclusive proof that the pair of degenerating nuclei are sister nuclei, but taken together the evidence is more impressive.

To the mycologist, concerned primarily with the life-cycle of *Allomyces arbuscula*, the significant fact in this account of meiosis is that the reduction division is located in the germinating zygote. To the cytologist, however, the attendant phenomena are quite as interesting, for they show that meiosis follows the same pattern here in the Phycomycetes as it does among the higher plants. The nuclei examined in this study are small and the chromosomes very small indeed, but the germinating zygotes are not as unfavourable material to work with as might be expected. There are certain advantages in diminutive nuclei that may very nearly outweigh their disadvantages. For instance, whole mounts can be used, and with complete zygotes it is possible to determine what is occurring in all parts of the cell at a given time.

The gametes.

VII. SUMMARY

1. The gametes are spherical to subglobose, uniciliate, uninucleate cells.
2. The female is $11\ \mu$ in diameter, its nucleus is $3.6\ \mu$ in diameter, and its colour is grey.

3. The male is $8\ \mu$ in diameter, its nucleus is $2.3\ \mu$ in diameter, and its colour is orange.

4. The motor apparatus of these gametes consists of a cilium, a rhizoplast connecting cilium and nucleus, and an intranuclear extension of the rhizoplast anchored to the nucleolus. The rhizoplast holds the nucleus close to the gamete membrane, and the intranuclear rhizoplast extension draws the nucleolus out into a beak.

5. Investing the nucleus closely is a nuclear cap, a bulky cytoplasmic structure of chondriosomal origin.

6. The lipid granules, small spherical cytoplasmic inclusions, are the carriers of the pigment that distinguishes the male and female gamete.

Conjugation.

1. The propinquity of male and female gamete is apparently achieved by chance, there being no long-range sexual attraction.

2. When both gametes are less than fifteen minutes old conjugation is rapid. When either is older conjugation becomes more and more protracted, finally ceasing at an age of approximately thirty minutes.

3. In conjugation the ciliated ends of the gametes must first be brought in opposition. This is accomplished by amoeboid movements on the part of the conjugants.

4. After this precise positional adjustment has been made the appressed membranes break down near the point of cilial insertion, forming a pore-like connexion between the two cells.

5. As the floor of this pore is depressed downward by a rush of cytoplasm, the male and female nuclei, which are suspended from the roof of the pore by their rhizoplasts, swing closer together.

6. As the nuclei approach each other the nuclear caps are brought in contact and thereupon fuse.

7. About the centrally disposed nuclei and their common nuclear cap the zygote is organized.

8. Nuclear fusion does not normally occur at this time, being delayed until zygote germination.

9. The abrupt mingling of cytoplasm incident to conjugation results in intense amoeboid activity of the zygote.

10. After some minutes of this activity the zygote rounds off and swims away with great vigour.

Zygote germination.

1. The extension of germ-tubes by the zygote is anticipated and conditioned by the dissociation of the nuclear cap. The fundamental adjustment in germination is thus made by a cytoplasmic structure of chondriosomal origin.

2. The first germ-tube gives rise to a tapering rhizoidal system. Only after this has become rather extensive does the hyphal germ-tube appear.

3. The important nuclear divisions associated with zygote germination are instituted when the zygote begins to bulge apically in the extension of this hyphal germ-tube.

Zygote germination and meiosis.

1. First meiotic division. In the division of the fusion nucleus the chromosome number is reduced from twelve to six.

2. Second meiotic division. In the divisions of the daughter nuclei the six chromosomes are split equationally, six chromosomes going into each of the four resulting nuclei. *a.* These divisions follow quickly upon the first. *b.* They are often simultaneous, always close. *c.* The anaphase chromosomes are smaller than those at a similar stage in the first division and are roughly one half their size. *d.* Of the four nuclei resulting from these divisions, two degenerate. These degenerating nuclei appear to be sister nuclei.

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EXPLANATION OF PLATES XVIII-XXII

Illustrating Dr. Hatch's paper on 'Conjugation and Zygote Germination in *Allomyces arbuscula*'.

Figs. 1-7, 12-15 fixed in a modified Schaudinn's fluid ($\frac{1}{3}$ strength) + 2 per cent. acetic acid, stained with iron-alum haematoxylin.

Figs. 16-27 fixed in a modified Schaudinn's fluid ($\frac{1}{3}$ strength) + 2 per cent. acetic acid, stained with iron-alum haematoxylin.

Figs. 28-53, fixed in $\frac{1}{3}$ per cent. solution of mercuric chloride + 2 per cent. acetic acid, stained with iron-alum haematoxylin.

PLATE XVIII

Figs. 1-15. The Gametes. $\times 2,100$.

Fig. 1. A female gamete.

Fig. 2. A male gamete.

Fig. 3. An amoeboid female gamete.

Fig. 4. An amoeboid male gamete.

Fig. 5. A biciliate female gamete.

Fig. 6. A biciliate male gamete.

Fig. 7. A female gamete showing the motor apparatus rather clearly.

Fig. 8. A female gamete vitally stained with Janus green.

Fig. 9. A male gamete fixed and stained by the Champy-Kull technique.

Fig. 10. A female gamete fixed and stained by the Meves technique.

Fig. 11. A female gamete fixed and stained by Cramer's osmic vapour method.

Figs. 12-15. Amoeboid female gametes.

c. = cilium; i.r.e. = intranuclear rhizoplast extension; L.G. = lipid granule; N. = nucleus; NO. = nucleolus; N.C. = nuclear cap; R. = rhizoplast.

PLATE XIX

Figs. 16-21. Conjugation. $\times 2,275$.

Fig. 16. A male and a female gamete. Note the proportionate sizes of the gametes, their nuclear caps, and their nuclei. The cilia twisted about each other and extending towards the top of the page can be seen with difficulty.

Fig. 17. Conjugants just after the initial collapse of the membranes. Male and female gametes are now joined by a cytoplasmic bridge between the ciliated ends of the cells. The point of cilia insertion can be determined by noting the position of the nuclei and particularly the position of the nucleoli. In the female (below) the nucleus and its nucleolus can both be made out without difficulty. In the male the outline of the nucleus is indistinct but the nucleolus can be seen quite clearly.

Fig. 18. Conjugants in which the connexion between the gametes has broadened permitting the two nuclei to swing together (male, left; female, right).

Fig. 19. Conjugants in which the male member, left, is larger than the female, right. The female must have emptied some of its bulk into the male.

Fig. 20. Conjugants in which the nuclei have been drawn closer together and the nuclear caps have begun to coalesce where their contiguous arms have come in contact.

Fig. 21. The planozygote. The nuclear caps have now fused and the zygote has rounded up, but the nuclei, despite their intimate association, still remain unfused.

PLATE XX

Conjugation. $\times 2,625$.

Figs. 23, 24, and 27 are drawn from the same conjugants photographed in Pl. XIX, Figs. 17, 18, 21 respectively.

Fig. 22. Male gamete crawling over a female.

Fig. 23. Conjugants just after the initial collapse of the membranes, as in Fig. 17.

Fig. 24. Conjugants in which the connexion between the gametes has broadened permitting the two nuclei to swing together, as in Fig. 18.

Fig. 25. Conjugants in a stage very similar to that of Fig. 19.

Fig. 26. A stage very like that shown in Fig. 20, except that the nuclear caps here have fused over a broader area.

Fig. 27. The planozygote, as in Fig. 21.

PLATE XXI

Figs. 28-36. Germination. $\times 2,275$.

Fig. 28. A zygote in which the dissociation of the nuclear cap has begun but is not far advanced. The nuclei, of necessity out of focus, are separated by the bulk of the nuclear cap, male above, female lower left.

Fig. 29. A stage in the dissociation of the nuclear cap. The propinquity of the nuclei is probably an inherited condition and does not represent progress toward an eventual nuclear fusion.

Fig. 30. A zygote in which the dissociation of the nuclear cap is complete enough for male and female nucleus to have come in contact. The comparative size of the nuclei and their nucleoli can be seen.

Fig. 31. A zygote in which the dissociation of the nuclear cap is nearly complete and in which nuclear fusion is in progress.

Fig. 32. Dissociation complete, nuclei so recently fused that the membrane has not yet rounded up and the male and female nucleoli have not yet fused.

Fig. 33. A zygote in which the chromatic material of the nuclear cap is further diffused and in which the nucleoli of the fusion nucleus have fused. The zygote has germinated (the narrow germ-tube can be vaguely seen in the lower left-hand corner).

Fig. 34. A germinated zygote in which the proportions of the fusion nucleus and nucleolus are fairly represented.

Fig. 35. A zygote in that stage of development at which the fusion nucleus divides. This is actually a metaphase figure. The persistent nucleolus can be easily seen but not enough chromosomes can be brought into the same focus to demonstrate their alinement on the spindle.

Fig. 36. A zygote in which germination has occurred (the germ-tube, very indistinct, lies between the gametes in the lower left-hand corner), but in which male and female nuclei have not yet fused.

PLATE XXII

Figs. 37-47. Germination. $\times 2,100$.

Fig. 37. A typical amoeboid with two intact cilia.

Fig. 38. An amoeboid zygote in which an amoeboid process has carried away the cilium that was attached to the female nucleus.

Fig. 39. An amoeboid gamete in which the connexion between the female nucleus and its cilium has been broken and the cilium sloughed off. The female nucleus in consequence has fallen away from the zygote membrane.

Fig. 40. A zygote that has come to rest, rounded up, and cast both of its cilia.

Fig. 41. A zygote in which the dissociation of the nuclear cap has clearly begun.

Fig. 42. A stage in the further dissociation of the nuclear cap.

Fig. 43. A zygote in which the dissociation of the nuclear cap is nearly complete and in which nuclear fusion is in progress.

Fig. 44. Zygote with a fusion nucleus in which the male and female nucleoli have not yet fused and in which a distinction can still be seen between the chromatic reticulum abutting on the male and female nucleolus.

Fig. 45. A germinated zygote in which the male and female nucleoli have fused.

Fig. 46. A planozygote in the formation of which an ordinary uniciliate male gamete fused with an extraordinary biciliate female. While the nucleolus of the male nucleus is drawn out into a single beak by reason of its connexion with a single cilium, that of the female is twice peaked because of its connexions with two cilia.

Fig. 47. A planogamete like the above only in this case there has been a precocious fusion of the male and female nuclei.

Figs. 48-53. Meiosis. $\times 2,625$.

Fig. 48. The first division; an oblique polar view of the metaphase plate, homologous chromosomes in pairs. The nucleolus, apparently central, probably lies in actuality on the far side of the spindle.

Fig. 49. The first division; a side view of the metaphase plate showing the actual separation of the homologous chromosomes. The nucleolus is visible behind the chromosomes.

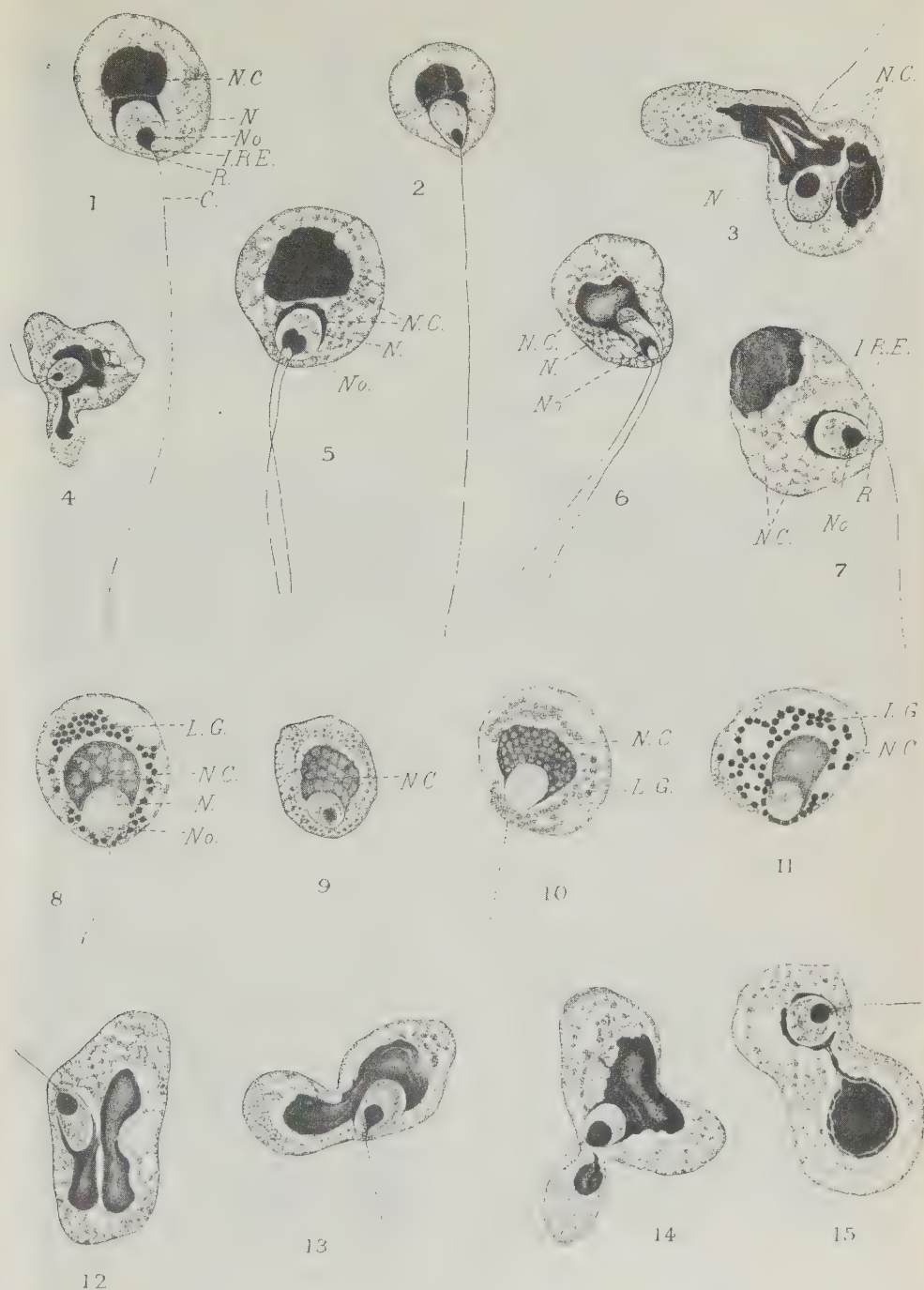
Fig. 50. The second division, simultaneous in both nuclei. Note how variable is the position of the nucleolus.

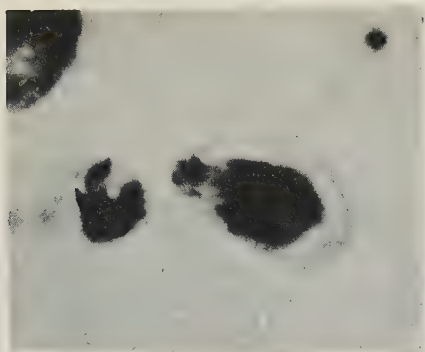
Fig. 51. The second division, also simultaneous in both nuclei, only in this case the two spindles are superimposed the one upon the other. One spindle is drawn in ink, the other in pencil. The pencilled nucleus has two nucleoli, one set in a pocket.

Fig. 52. Second division, also simultaneous. To avoid confusion the chromosomes are drawn in only one nucleus.

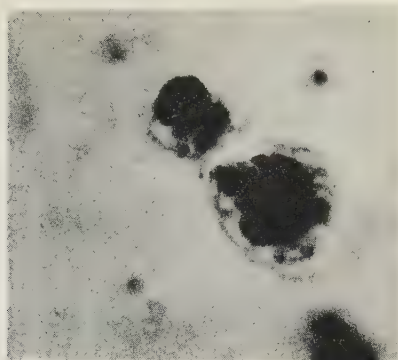
Fig. 53. A second division that is not simultaneous. Inset is a drawing of the same nucleus figured in the zygote itself only at a slightly different focus.

A comparison of the chromosomes in the first division (Figs. 48 and 49) with those in the second division (Figs. 50-3) indicates that the chromosomes in the second division are smaller than, possibly one-half as large as, the chromosomes in the first division.

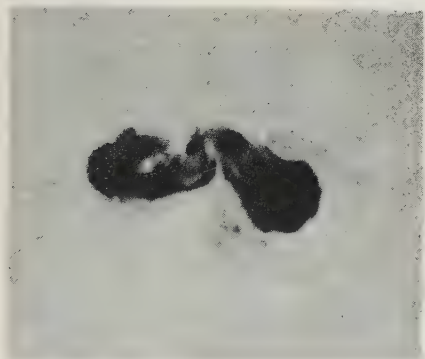




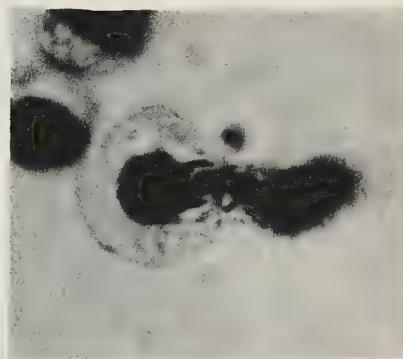
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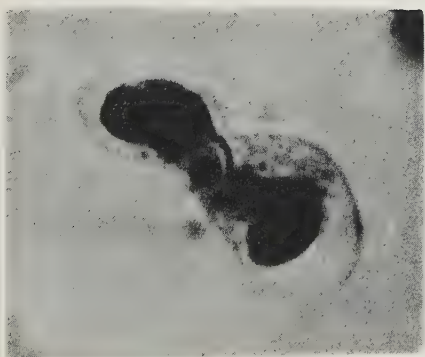
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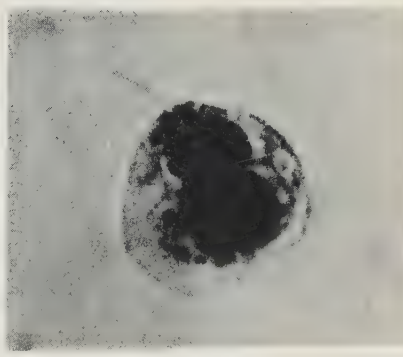
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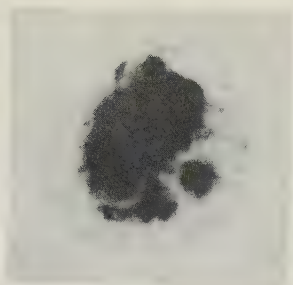


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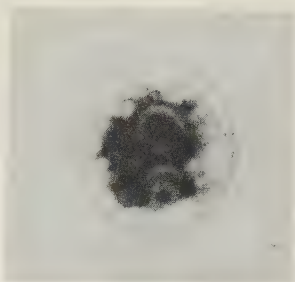


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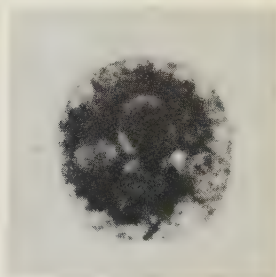
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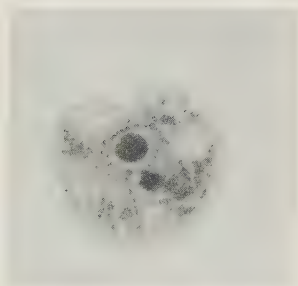
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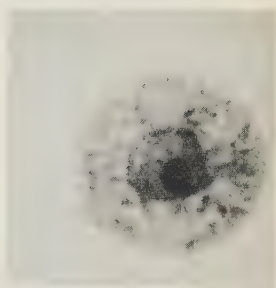
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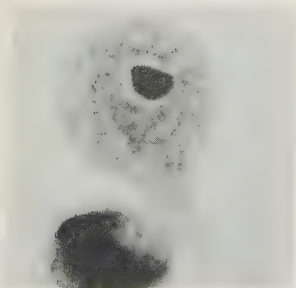
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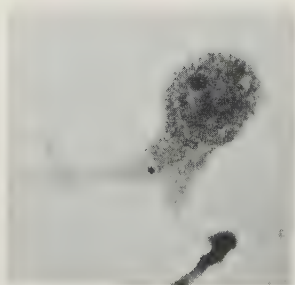
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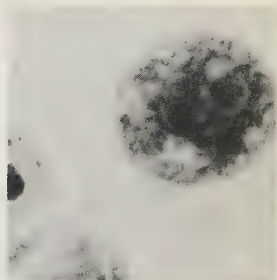
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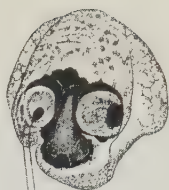


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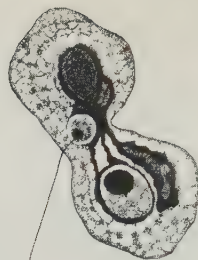
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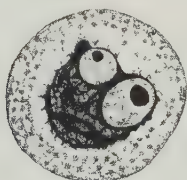
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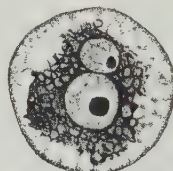
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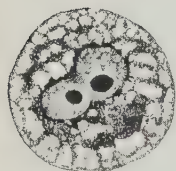
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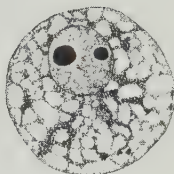
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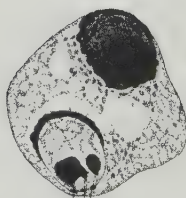
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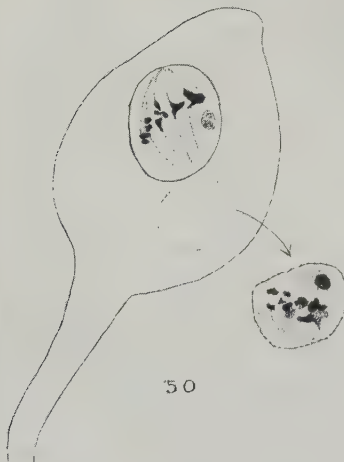
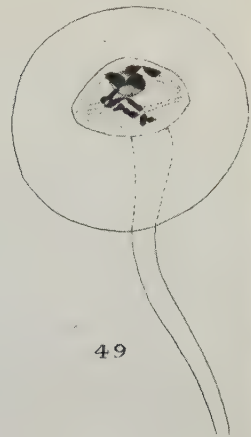
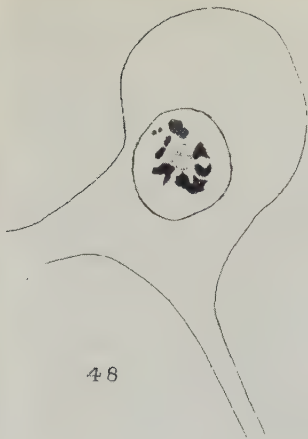
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Differential Reactivity of the Chromosomes

BY

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With Plates XXIII and XXIV and eight Figures in the Text

I. THE PROBLEM

IN recent years the application of a variety of new methods of experimental treatment and fixation has enabled us—although perhaps not to the satisfaction of all—(cf. Addendum, p. 624), to disentangle fact from fiction in the story of the mitotic cycle. It has also revealed several kinds of heterogeneity between different parts of the same chromosome, heterogeneity which is to be related to differences sometimes of genetical function and sometimes of mechanical function. We are concerned in the following account with applying new methods of pre-treatment and fixation to the attack on these problems.

2. FIXATION AND STRUCTURE

The study of the internal structure of mitotic chromosomes has been largely carried out with root-tips embedded after fixation and prepared in sections. The method of staining (with haematoxylin or gentian violet) is not of primary importance in regard to the structure seen with this method. The structure depends almost entirely on the fixative. The question then arises as to whether any internal structure observed by these methods at metaphase or anaphase has any relation to the structure which is inherent in the developmental changes of the living chromosome; whether, in fact, the artefacts seen are significant and specific or merely irrelevant. This question may be answered by the following comparative tests.

In root-tips fixed with medium Flemming solution and stained with gentian violet the chromatids are optically homogeneous under correct illumination. If examined or photographed out of focus or with infra-red light to which the microscope is not adjusted, a single or double light-band runs down the middle of the chromatid (Pl. XXIII, Fig. 3). The same effect may often be obtained when a fixation in non-acetic Flemming gives the appearance of a hollow tube to the chromosome, which then in optical section seems to consist of two parallel threads. These threads may even seem to

cross over one another at secondary constrictions (Pl. XXIV, Fig. 13). These are the first types of artefact or optifact and they have frequently been illustrated (cf. Darlington, 1935). A second and more widely discussed artefact is the one that has been variously interpreted as due either to two threads coiling round one another inside a cylindrical matrix of which the chromatid is largely composed, or to mere vacuolation of the chromatid, that is, to the formation of bubbles of differential refractivity. This type of artefact can be produced in its clearest form by fixation with acetic alcohol. It is then found that the bubbles are sometimes fairly regular, that is, of equal size and at equal distances apart in the middle of the chromatid. More usually, however (and in the same preparations), the vacuoles are of unequal sizes and irregularly spaced (cf. Pl. XXIII, Fig. 1). Similar results are sometimes given by medium Flemming (Pl. XXIV, Figs. 14, 15). When metaphase chromosomes are compared in different species, we find that the size and arrangement of the vacuoles depend on the diameter of the rod under consideration (cf. Text-figs. 1 and 2) and cannot therefore be taken seriously as evidence of the living structure. Moreover, they are inconsistent within the same chromosome and inconsistent therefore with any regular interpretation of the structure. Such fixations indeed show that the vacuoles can be interpreted only as irrelevant artefacts.

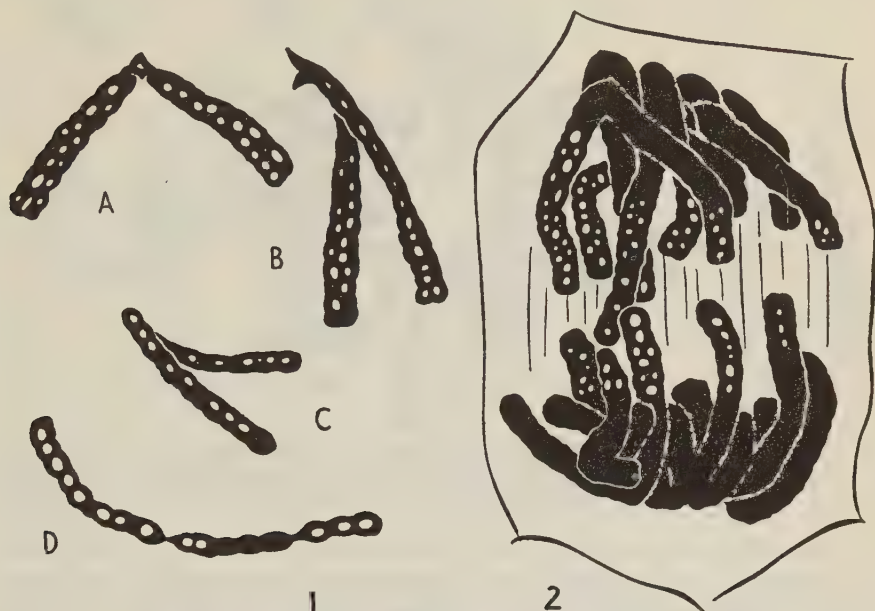
We quote these examples (which might be multiplied with the use of different methods) merely to show the difficulties which arise from the long-established procedure which leads to the paraffin block and the microtome.

Different results, however, are obtained when we use special tissues and special methods of fixation and pre-treatment chosen for the rapidity and uniformity of their action. There is no mitotic cell in the higher plants more readily fixed than the generative cell in the rapidly growing pollen tube. Fixed with simple acetocarmine it shows the same spiral structure that has been revealed experimentally by Kuwada and others at meiosis. Furthermore, these spirals can be counted, and they can be seen to have uncoiled at telophase in the same way as they do in experiment when the pH of the medium is changed (Upcott, 1936).

At telophase in root-tip smears the slight uncoiling of the internal spirals can be recognized as in the pollen tube. When, however, a nucleus is opened out in smearing, the coils are stretched and their structure becomes clearer, very much as in pre-treated meiotic metaphases. It is then seen that the spirals pulled apart in this way are about half the thickness of the anaphase chromatid, and further that they give indications of containing within themselves a further minor spiral like that found in meiosis (Pl. XXIV, Figs. 16-18). Further study must be devoted to examining this fundamental property.

These observations show that the secret of determining internal structure is the rapid fixation of cells whose walls have been removed by special treatment and without the processes of hardening and embedding which lead to the variable coagulation of living structures.

To test these methods we have taken root-tips of a plant of *Paris polyphylla* L. (obtained from the Edinburgh Botanic Garden, and corresponding with the illustration in *Pflanzenfamilien*, ii. v, Fig. 83) and prepared them for comparison with different fixatives, some for embedding and cutting as sections and some as smears.



TEXT-FIG. 1. Metaphase (A and B) and anaphase (C and D) chromosomes in *Hyacinthus orientalis*. Smear, fixed in acetic alcohol. Photograph, Pl. XXIII, Fig. 1. ($\times 2,700$.)

TEXT-FIG. 2. Anaphase in *Trillium sessile*. Smear, fixed in medium Flemming. Photograph, Pl. XXIV, Fig. 15. ($\times 1,800$.)

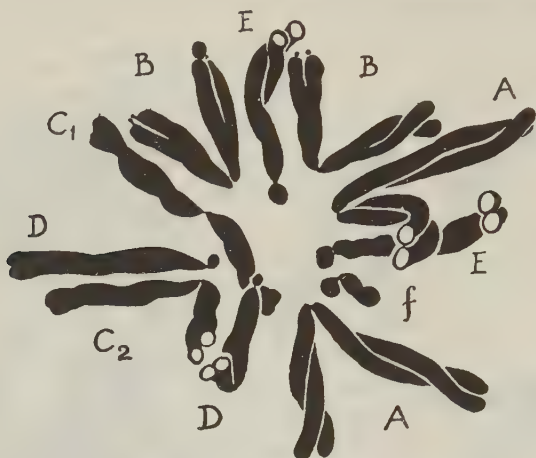
3. THE SPECIAL SEGMENTS IN PARIS

An ordinary permanent fixation in 2 BE (La Cour, 1937) gives the result illustrated in Text-fig. 3. It shows three pairs of chromosomes with submedian centromeres and two pairs with subterminal centromeres. There is in addition one extra fragment chromosome. The five distinct pairs can be made out, although the distinction between A, B, and C is not very sharp. The B type varies in showing a secondary constriction and a trabant.

Four preparations¹ made from two fixations on the same day in March 1937 showed a characteristic differential reaction not previously observed in chromosomes. These preparations were pre-treated with nitric acid vapour and fixed with non-acetic Flemming and stained by the Feulgen smear method described in the appendix. It must be clearly understood, however, that although the same method used on other occasions later in the year has

¹ For two of these we are indebted to Dr. L. Husted of the University of Virginia, who was working at the time in this laboratory.

given similar results, it has not given the completely constant and sharply differential behaviour to be described from these four preparations. It seems, therefore, that the condition of the plant, possibly depending on temperature, is concerned with the reaction. Fixations giving incomplete differentiation

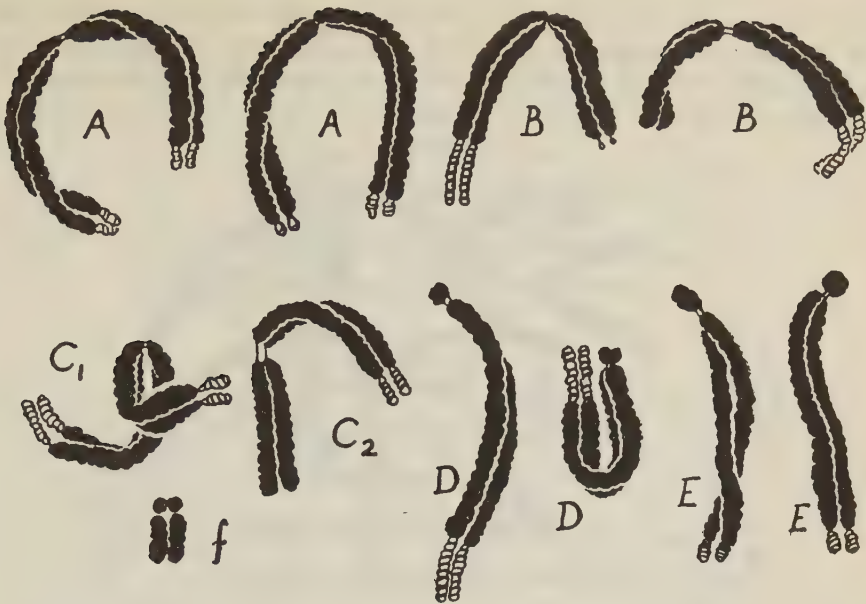


TEXT-FIG. 3. Metaphase in *Paris polyphylla*, $2n = 10 + f$, section, fixed in 2 BE, after pre-treatment with sugar solution; stained with gentian violet. ($\times 1,800$.)

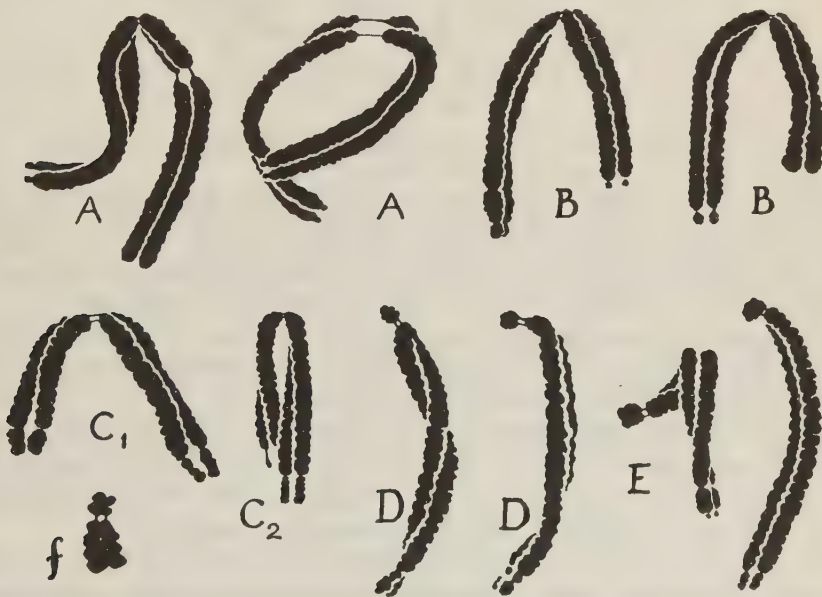
show in the minimum effect merely a narrower chromatid in the special segments; in an intermediate effect, lower staining, secondary constrictions, and less regular outline. It should be noticed that with a vacuolar fixation the vacuoles do not show in the narrower special segments. Later we shall consider these intermediate fixations.

The differential reaction consists in particular and constant parts of each chromosome being coiled in a finer thread of smaller diameter than the rest of the thread. Successive coils remain the same distance apart. In consequence of this the number of coils in these special or differentially reactive regions can usually be counted when they are undamaged in smearing (cf. diagram, Text-fig. 8). Possibly on account of the reduced thickness of the chromosome thread it is less deeply stained. The relationship of the thin thread segments to the rest of the chromosome is very much like that of a meiotic chromosome which has been contracted to show its major spirals with nitric acid vapour to a normally fixed chromosome. In *Paris* the difference is a difference in the reaction of different parts of the same chromosome to the same treatment.

The differential segments being of characteristic length for each chromosome arm enable us to distinguish each chromosome type in every cell (Text-figs. 4, 6, and 7). We can then say that the lengths of the differential reactive segments are the same when showing their special reaction as in other preparations similarly treated when not showing it (Text-fig. 5). The lengths of the special segments vary from a fraction of a coil to a maximum of eight coils



TEXT-FIG. 4. Metaphase chromosomes of *P. polyphylla*, showing differential regions after pre-treatment with sugar solution and nitric acid vapour, non-acetic Flemming fixation and Feulgen staining. Photograph, Pl. XXIII, Fig. 9. ($\times 1,800$.)



TEXT-FIG. 5. The same material and treatment as Text-fig. 4, but from a later preparation not showing constant differential regions. Photograph, Pl. XXIII, Fig. 6. ($\times 1,800$.)

(in the long arms of B and D), and this again agrees with the number of coils found in the entirely normal larger arm of the fragment, namely, six. That is, the number of coils is proportional to length both in normal and special segments and shows a width of coil of about 0.6μ or half the diameter of



TEXT-FIG. 6. Anaphase from the same preparation as Text-fig. 4.
Photograph, Pl. XXIII, Fig. 8. ($\times 1,800$.)

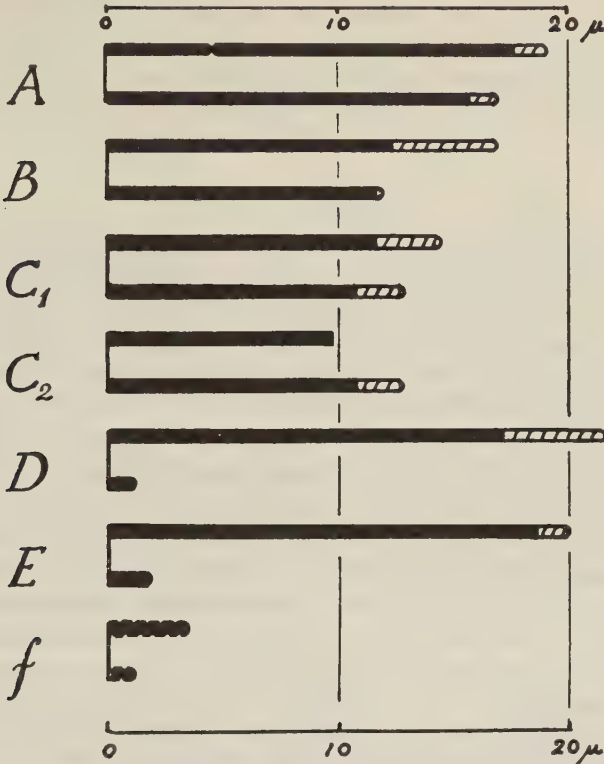
the chromatid. The same relationship was found in *Tulipa* and indicates a compact coil of circular cross-section. The coiling cannot be made out for a great enough length in the normal parts of the chromosome to determine their number accurately in an arm longer than that of the fragment.

The variable appearance of a knob somewhat different from the ordinary trabant on the short arm of the B (also found in normal preparations) may be due to a very short differentially reactive region.

Owing to the constancy of the special regions we can distinguish between the two homologous chromosomes of the c type. C_2 has lost the end of the longer arm found in C_1 and with it the special segment. Whether the origin of the fragment is connected with this change we cannot tell.

The special segments show their properties inside the prophase nucleus, although the smear method is not favourable to the study of this stage. They also maintain their properties with complete regularity at anaphase (Pls. XXIII and XXIV, Text-figs. 6 and 8).

When we come to consider the positions of the special segments we see that they are confined to the ends of the chromosomes and, in fact, to the ends of those arms which are more than $12\ \mu$ long. All arms longer than this have a special segment, none of those shorter have any. Moreover, although



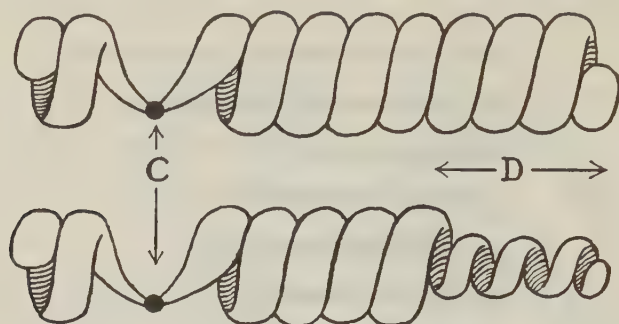
TEXT-FIG. 7. Diagram showing the approximate lengths of all the chromosomes of *Paris polyphylla* together with their differential regions and with the number of their coils. These are also shown in the fragment. The long arm of D has about 29 coils in its normal segment, making 37 in the whole arm. The centromere joining the two arms is indicated by a line on the left-hand side. The chromosomes are the same length when unstretched, whether they show the differential reactivity or not.

all the long chromosomes have at least one special segment, the supernumerary short chromosome has none. The possibility of having a special segment or of showing a differential reaction, therefore, seems to depend on distance from the centromere. Again it does not depend on this alone but is specific to particular parts of the chromosome.

4. THE CONDITIONS OF DIFFERENTIAL REACTIVITY

Broadly two kinds of differential reactivity are known within the chromosome thread, apart from the centromere itself. The first is the kind described by Heitz as due to heterochromatin. It expresses itself probably in three

ways. First, by the special parts remaining contracted and deeply staining longer than the rest in a mitotic telophase. Secondly, by their remaining diffuse or unstained, as in the salivary glands of *Drosophila*. Thirdly, if this indeed be a related property, by a precocious condensation during prophase,



TEXT-FIG. 8. Diagram showing the appearance of the same chromatid end with and without the differential reaction of the three distal coils. C, the centromere; D the differential region.

especially of meiosis in animals. When the Y chromosome in *Drosophila* was found to have this property at the telephase of mitosis, it became evident that it was correlated with genetic inertness (Heitz, 1935).

Another kind of differential condensation which may be inherent in the living chromosomes or may be a fixation reaction is the precocious condensation of the proximal parts of the chromosomes in *Agapanthus*, *Zea Mays*, and *Fritillaria* in the prophase of meiosis. This behaviour may be inherent in the particles concerned or it may depend on their position near the centromere. The second possibility is more likely (Darlington, 1937).

Naturally we want to know whether the behaviour of Paris has anything to do with these previously known types of reaction. The relationship with the centromere suggests an analogy with *Agapanthus*. The exact differentiation of two kinds of segment suggests an analogy with *Drosophila*. Why then should the differential regions be entirely in distal parts of the chromosomes? We do not know, but since species of Paris previously studied have proximally localized chiasmata (cf. Darlington, 1937, p. 110) it is possible that these are segments which have become inert through lack of crossing-over in them. The loss of the differential region in c_2 would then become intelligible.

Finally, the question arises as to why in most fixations the differential regions should be variable in appearance, while in a few they are constant. In the first place we must suppose that there is no difference in the behaviour of the normal and differential regions in the living chromosome. It seems possible then that the differential reaction is an all-or-nothing reaction and that under most conditions the chromosomes are very near to its threshold, some like the A's in Text-fig. 5 being below while others like the C's are above,

others again like the B's being so close as to show the positions of the differential regions by secondary constrictions. Such variations suggest that the character of the differential regions may differ in different chromosomes (as for example in the proportion of inert genes) although their differences are concealed in the fixations which give constant results.

Further study by these methods should enable us to distinguish more exactly between the physiological and mechanical aspects of differential reactivity indicated by our present observations.

5. SUMMARY

1. A variety of different structures may be inferred in the chromosome from the results of a variety of different treatments. The correct inference must therefore be that which is consistent in itself and consistent as between different organisms and successive stages. With special treatment the mitotic chromosomes can be uncoiled like the meiotic and show a similar simple spiral, possibly containing a minor spiral within it.

2. Special treatment enables us in *Paris polyphylla* to detect a new and constant differential reactivity within the chromosomes. From one to eight distal coils of the longer chromosome arms are differentially contracted during mitosis. This artefact is specific for each chromosome type and is probably correlated with the genetic function of the differential regions.

APPENDIX ON TECHNIQUE

1. *Pre-treatment.*

Root-tips were slit longitudinally at the apex with a sharp scalpel and wet in a 3 per cent. cane sugar solution. They were then held over a jar containing concentrated nitric acid for 10–45 seconds before placing in the fixing solution. This method was used before both the smearing and the embedding treatments.

2. *Fixatives.*

(1) Acetic Alcohol :

3 : absolute alcohol.

1 : glacial acetic acid.

(2) Medium Flemming :

30 : 1 per cent. chromic acid.

10 : 2 per cent. osmic acid.

25 : 5 per cent. acetic acid.

(3) Non-acetic Flemming :

As above without acetic acid.

(4) La Cour's 2 BE :

Cf. La Cour 1937.

3. *Smear Technique* (adapted from Heitz's Nucleal-Quetschmethode, 1936).
 1. Fix (1 to 4).
 2. Hydrolyse in normal HCl at 60° C.
after (1), 6 minutes.
 (2) and (4), 16 minutes.
 (3), 25 minutes.
 3. Feulgen stain for 3 hours.
 4. Macerate in 45 per cent. acetic.
 5. Mount under pressure.
 6. Heat intermittently.
 7. Separate cover and slide in equal parts xylol, glacial acetic acid and absolute alcohol, and leave in mixture 5 minutes after separation.
 8. Xylol-absolute alcohol, equal parts: two changes.
 9. Xylol.
 10. Mount.

N.B. Gentian violet or haematoxylin may be used in place of Feulgen with similar results, if followed by appropriate treatment. The smearing sometimes stretches the chromosomes and secondary constrictions then appear which would otherwise be invisible (A, Text-fig. 5; c, Text-fig. 6).

4. *Sections (Paris polyphylla).*

Fixatives 3 and 4, sections 36 μ thick, stained gentian violet.

ADDENDUM

A further discussion of the problems dealt with in the present article will be found in the following three articles under the title 'Structure of Chromosomes'. Gates, R. R., 1937. *Nature*; 140, 1013. Darlington, C. D., 1938. *Nature*; 141, 371. Gates, R. R. and Mensinkai, S.V., 1938. *Nature*; 141, 607.

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 UPCOTT, M. B. 1936: The Mechanics of Mitosis in the Pollen-tube of *Tulipa*. *Proc. Roy. Soc. B.* cxxi. 207-220.
 — 1937: The External Mechanics of the Chromosomes, VI. The Behaviour of the Centromere at Meiosis. *Proc. Roy. Soc. B.* cxxiv. 336-361.

EXPLANATION OF PLATES

Illustrating Dr. Darlington's and Mr. La Cour's paper on 'Differential Reactivity of the Chromosomes'.

Plates XXIII and XXIV. Microphotographs, some of which represent cells illustrated in the text.

PLATE XXIII.

Fig. 1. *Hyacinthus orientalis*, root-tip smear (no pre-treatment), acetic alcohol fixation, Feulgen stain. Metaphase and anaphase. Vacuolar artefacts. Cf. Text-fig. 1. ($\times 1,600$.)

Fig. 2. The same, but with medium Flemming fixation, showing optically homogeneous structure. ($\times 1,600$.)

Figs. 3 and 4. *Fritillaria ruthenica*, meiosis in pollen mother-cells, aceto-carmin smear. Photographs with low aperture of illumination in focus (4) and out of focus (3) showing two kinds of optifacts. ($\times 1,600$.)

Fig. 5. *Paris polyphylla*, root-tip. Pre-treated smear, non-acetic Flemming fixation, Feulgen stain. March preparation. Anaphase of mitosis showing differential regions. ($\times 1,000$.)

Fig. 6. The same at metaphase. Summer preparation, cf. Text-fig. 5. ($\times 1,300$.)

Figs. 7 and 8. The same at metaphase and anaphase. March preparations showing differential regions (Text-fig. 6). ($\times 1,100$.)

Fig. 9. The same (Text-fig. 4). ($\times 1,300$.)

PLATE XXIV.

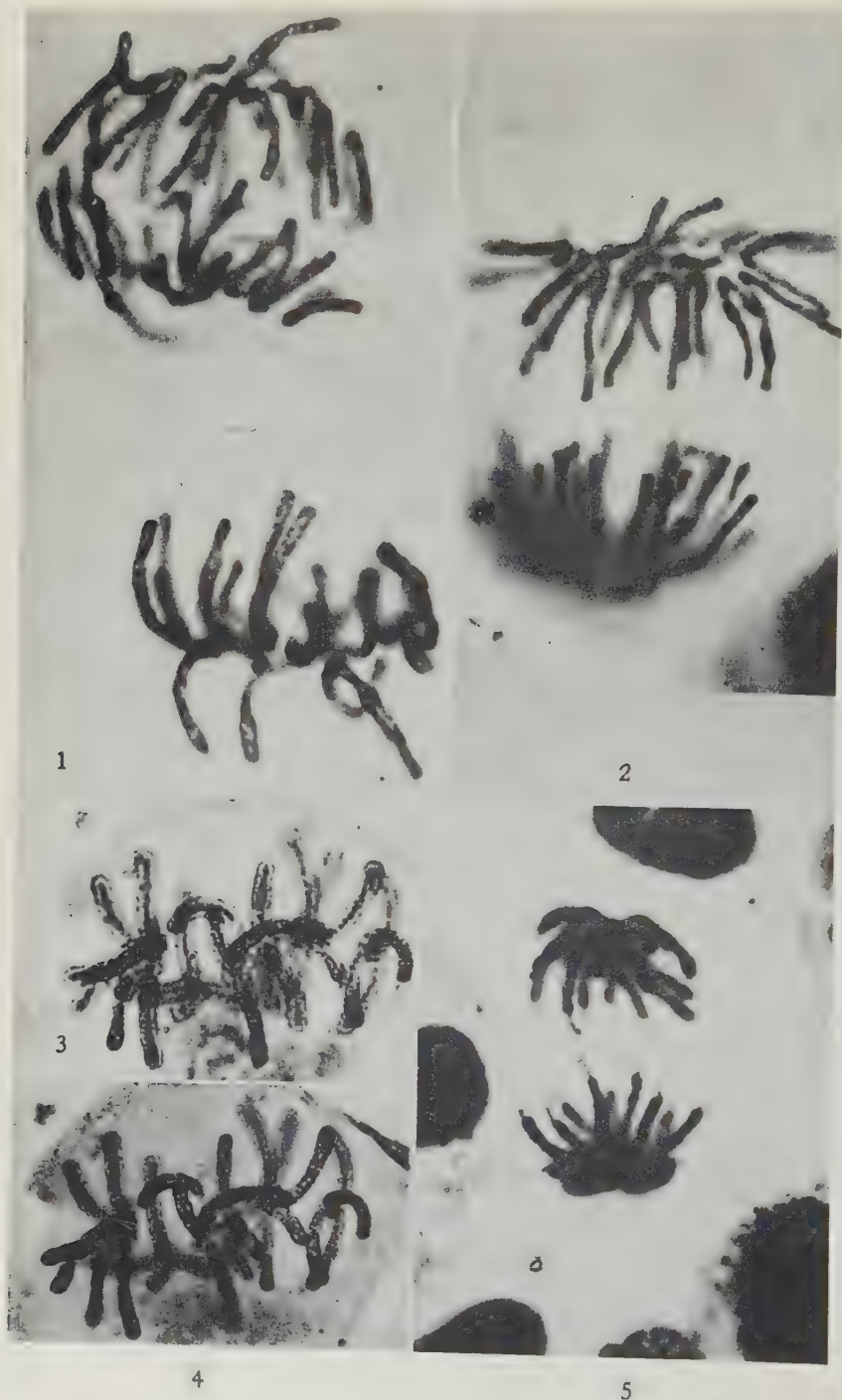
Figs. 10 and 11. The same: anaphase $\times 1,500$. Metaphase, $\times 3,000$.

Figs. 12 and 13. Non-acetic Flemming fixation of the same plant, sections, stained with gentian violet. Differential regions variable. Granular and tubular artefact structure. ($\times 1,600$.)

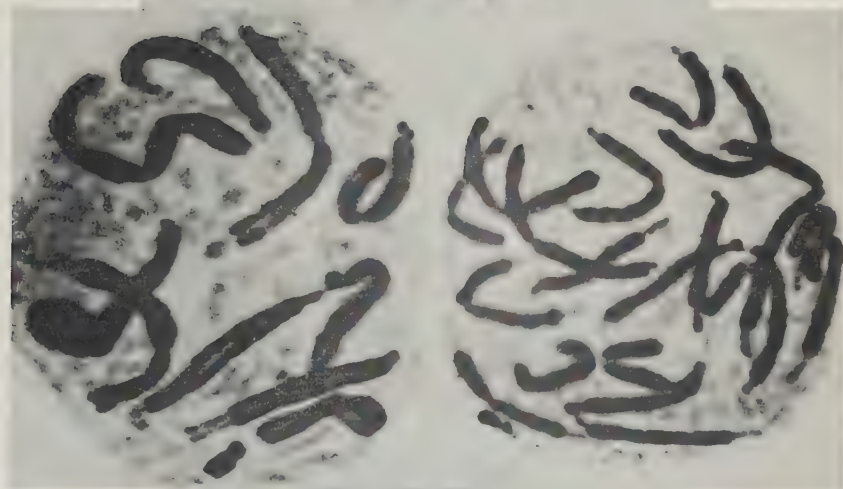
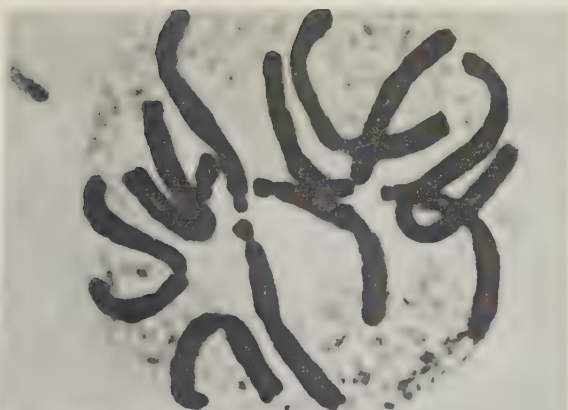
Figs. 14 and 15. *Trillium sessile*: non-pre-treated smear fixed with medium Flemming, Feulgen stain. Vacuolar artefacts. ($\times 1,600$.)

Figs. 16 and 17. *Paris polyphylla*, root tips. Sections fixed in non-acetic Flemming and stained in gentian violet, showing the development of relic spirals from internal spirals in telophase. ($\times 1,200$.)

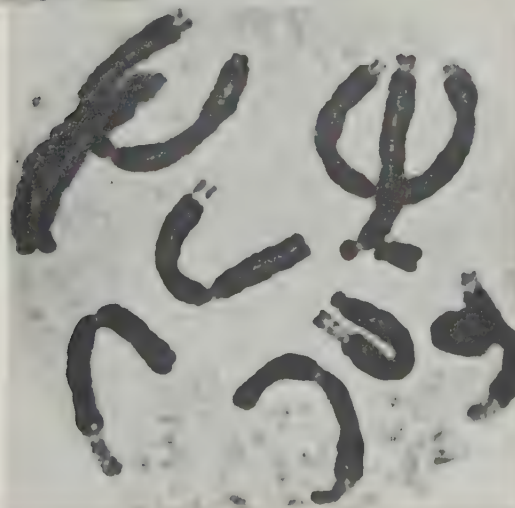
Fig. 18. Smear preparation of the same stage, pre-treated, fixed in non-acetic Flemming, stained Feulgen. A torn nucleus exposing internal spirals. ($\times 1,500$.)



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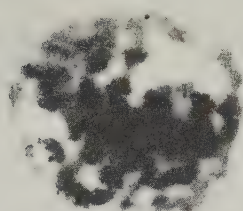
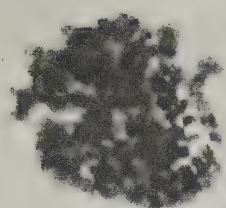


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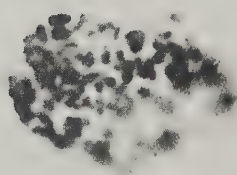
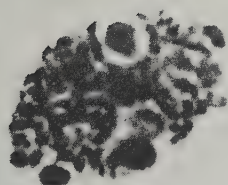
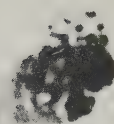
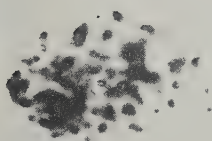


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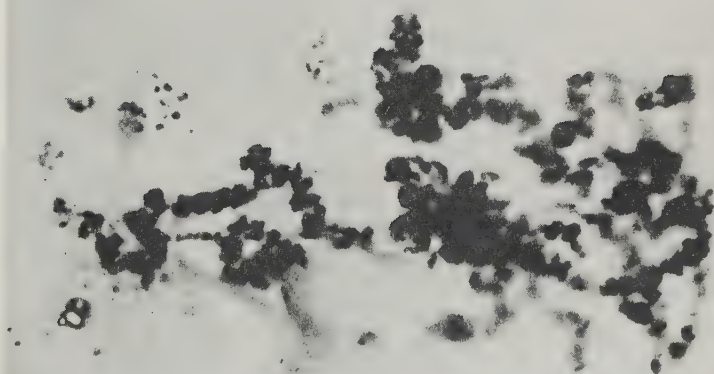




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17



18

Transpiration and Pressure Deficit

V. The Direct Measurement of Transpiration under Pressure by a Weighing Method¹

BY

F. M. HAINES, Ph.D.

With five Figures in the Text

INTRODUCTORY

PREVIOUS investigations by potometer methods (Haines, 1935, 1935*a*), eosin methods (Haines, 1935, 1935*a*), and a thermopile method (Haines, 1936) have shown that a pressure deficit applied to a cut branch by the pressure cylinder method brings about a considerable decrease in the rates of absorption and transpiration. The magnitudes of these effects for a wide range of pressure deficits produced in this way have also been determined. In all the experimental methods recorded so far, however, the changes in transpiration rate have been computed from other determinations owing to the difficulty of devising a satisfactory means of measuring transpiration in the cylinder directly by the amount of water vapour given off. Confirmation of the results by direct measurement of transpiration is therefore evidently desirable. Moreover, for the purpose of estimating the effects of similar deficits produced under natural conditions the experimental methods so far employed are inadequate in that they do not permit of any type of control experiment by which it can be determined in how far the effects produced by increase of pressure in the cylinder are due to the deficits as such (i.e. to the difference in pressure between the atmosphere at the leaf surface and the tracts inside the plant) and in how far to the actual increased pressure at the leaf surface *per se*, which alone would be expected to retard the diffusion of water vapour away from the leaf and so cause a reduction in the evaporation rate. In other words, it has not been possible to estimate the effect of moving all the pressure values up the scale as compared with those existing in natural circumstances. By the method utilized in the present series of experiments the transpiration rates are measured directly by weighing the water vapour given off and at the same time control experiments are possible under conditions of increased pressure at the leaf surfaces but with no deficit, the pressure in the tracts being similarly increased. It will be noted from what follows that the direct determinations of transpiration rates afford results

¹ From The Botanical Department, Queen Mary College, London.

which are in good agreement with those obtained by the earlier methods of estimation, thus confirming the values of the effects of deficits produced by the pressure cylinder method as given in previous communications (*loc. cit.*). On the other hand, the newly devised control experiments have shown that some of the previous conclusions on the effects of similar deficits produced under natural conditions, where the deficits exist without any increased pressure at the leaf surfaces, must be considerably modified.¹

APPARATUS AND METHOD

The general arrangement of the apparatus used in the present series of experiments is shown diagrammatically in Fig. 1. The general method of experiment will be described first and the details of the construction of certain components of the apparatus will be given later.

The method depends upon passing dry air over the plant under pressure, collecting the water vapour given off in a drying tube, and weighing. A cut branch was enclosed in the cylinder, J (Fig. 1), with its butt end (except in control experiments) passing out through the base as in the earlier series (Haines, 1935, p. 224), the calcium chloride in the cylinder of course being omitted. To create a pressure deficit of any desired value air could be pumped into the cylinder by means of a compressor driven by a $2\frac{1}{2}$ h.p. electric motor. The ingoing air was dried under pressure by passing through a specially designed steel drying tube (Fig. 1, F) containing anhydrous calcium chloride. (The details of construction of this tube are given below, p. 631.) By control experiments with no plant in the cylinder the quantity of water vapour remaining in the air after passing over the drying agent in the steel drying tube at the speeds used was found to be negligible. To ensure complete scavenging of the transpired water vapour the experimental branch in the cylinder was placed under an inverted 'collecting funnel', L, the upturned stem of which was connected with the outlet tube, M. The outgoing air (still under pressure) passed from the cylinder via the tube, N, to a specially constructed outlet valve, O, which allowed air to pass it only when a certain pressure was reached in the cylinder. The pressure maintained in the cylinder was thus decided by the setting of the valve. This could be regulated in two ways. It could either be set by varying the tension in an adjustable spring so that a constant pressure was maintained in the cylinder automatically, or be adjusted directly by hand independently of the spring control.

¹ As already indicated (Haines, 1936, p. 21) it appeared from preliminary experiments with the method to be described that the effects obtained in pressure cylinder experiments were not to any marked extent due to the increased pressure at the leaf surfaces as such, but were almost entirely due to the deficits thus created when the tracts remained at atmospheric pressure. Further work has shown, however, that the results of these preliminary experiments were fallacious owing to too slow an air stream through the apparatus and the absence of the collecting funnel, L (Fig. 1), in the cylinder to ensure complete scavenging of the water vapour. The importance of these points was only recognized later as a result of a larger number of preliminary experiments.

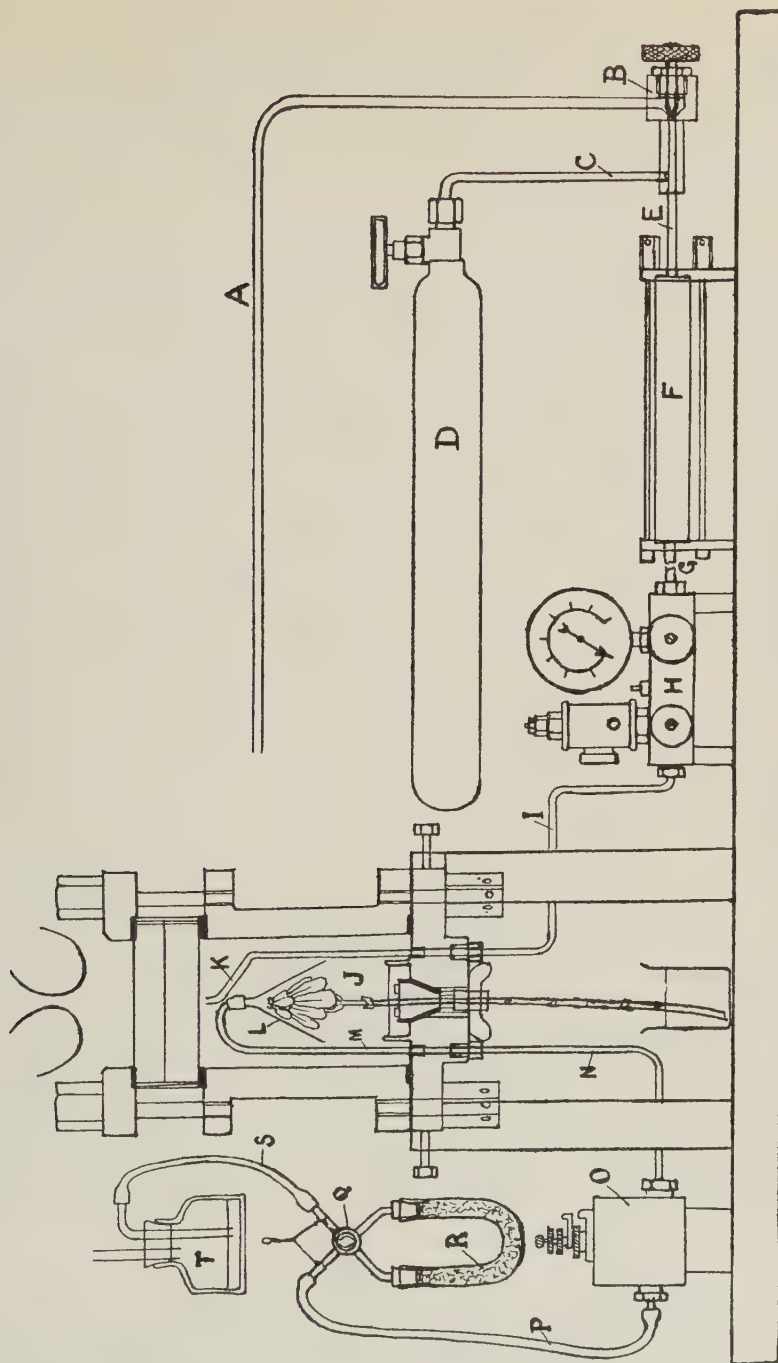


FIG. 1. General arrangement of the apparatus for experiments by the weighing method. For explanation see text, p. 628. For further details cf. Haines, 1935, Fig. 2, p. 220.

In general, manual control was more satisfactory at the lower pressures and the automatic control at the high pressures and for longer experiments. In this way dried air could be passed over the plant at any desired pressure up to approximately 25 atmospheres, which was more than double that necessary to reverse the direction of conduction in any of the plants investigated. After leaving the outlet valve, the air, containing transpired water vapour and now at atmospheric pressure, was passed through a glass U-tube, R, containing anhydrous calcium chloride, in which the transpired water was weighed in the usual way. By a special four-way tap, Q, having two opposite tangential cuts on a solid key and therefore capable of connecting adjacent pairs of leads in two different ways, the drying tube could be cut out of the circuit and by-passed through the tap or re-intercalated at will. Control experiments with further drying tubes showed, that with fresh charges of calcium chloride and the rates of air current used, over 98 per cent. of the total moisture was absorbed in the first tube. A single tube was therefore considered to give sufficiently accurate results for the present purposes. After passing through the drying tube the air was allowed to bubble through a very shallow layer of concentrated sulphuric acid in the bottle, T, which served to prevent leakage of atmospheric moisture back to the drying tube and could also be used (as will be described below) as an indicator of the speed of the air current.

The method, it will be realized, depends upon the pressure-reducing action of the outlet valve, as a result of which, although the air may be circulated over the plant at considerably increased pressure, the transpired water vapour may be collected and weighed with normal apparatus at atmospheric pressure in the usual way. It possesses the great advantage that it readily allows of control experiments being performed with the plant entirely enclosed within the cylinder. With this method the butt end only need pass out through the base for the purpose of creating a deficit when required, this being no longer necessary for the purpose of making the measurement, as is the case with the eosin and potometer methods. By placing the cut end of the branch in a small beaker of water also enclosed entirely within the cylinder, the transpiration rate may be measured with any desired pressure at the leaf surfaces but with no pressure deficit, the pressure within the tracts being automatically increased to the same extent. Comparison of the results of such control experiments with those of experiments in which the butt passes through the base of the cylinder to create a deficit readily shows the extent of the effect of the deficit as such, independently of any direct effects of the increased pressure at the leaf surfaces.

Details of the apparatus.

The pressure cylinder. The details of the pressure cylinder itself have been given in an earlier paper (Haines, 1935, p. 219), the only additions for the present experiments being the inlet and outlet tubes, K and M, and the collecting funnel, L (Fig. 1), within the cylinder to cause the outgoing air

to circulate more efficiently over the plant. The disposition and action of these will be sufficiently evident from the figure (Fig. 1). The tubes are of brass with an internal diameter of approximately $\frac{1}{8}$ inch and are screwed into the base of the cylinder. The outlet tube, M, is connected to the collecting funnel, L, by a rubber connexion. The diameter of the mouth of the funnel is 10 cm.

The drying tube. The steel drying tube for drying the ingoing air under pressure is specially constructed to allow rapid recharging with anhydrous calcium chloride and to withstand pressures up to 120 atmospheres, to which it might be exposed by inadvertent opening of the valves in the wrong sequence (especially when, as in the earlier experiments (1935-6), a high-pressure air cylinder is being used as a source of air supply instead of the compressor). The details of construction are shown in Fig. 2. It consists in a cylindrical body, C, of steel tubing, $\frac{1}{8}$ inch thick and with an internal diameter of 1 inch, and two ends, AA, of $\frac{1}{2}$ inch steel plate held in position by four $\frac{1}{2}$ inch steel tie-rods, BB, secured at one end by the nuts, FF, which are easily and rapidly removable by means of a tommy-bar. The ends, AA, are recessed for the reception of the body to a depth of $\frac{1}{8}$ inch, the recesses containing thin rubber washers, DDDD. Immediately below and flush with each recess is a small rest, E, shaped above like the recess itself. On these rests the body remains supported when the ends are drawn apart to take out the body for recharging. The rests also save considerable time when replacing the body after charging. After refilling with calcium chloride a pad of cotton-wool and a gauze partition mounted on a collar fitting the tube are placed in each end of the body and the latter dropped upon the rests. The nuts are then tightened, pulling the ends home against the body to form an air-tight joint in the recesses.

The outlet valve. The detailed construction of the outlet valve is given in Fig. 3. The valve consists of a solid steel block, AA, measuring $4\frac{1}{4}$ by $3\frac{3}{4}$ inches by $1\frac{1}{2}$ inches thick. The block is provided with two connexions, of which B serves as inlet from the pressure cylinder and C acts as outlet to the glass drying tube, R (Fig. 1). To pass from B to C the air has to pass upwards through a vertically drilled hole in the region, DD, acting as valve seating, and raise the valve, E. The valve, E, is made solid with a brass piston, F, accurately fitting a vertical cylindrical recess, G, in the centre of the block in which it can move up and down to open and close the valve. Since the pressure beyond the valve is scarcely above that of the atmosphere, this device effectively prevents any escape of air upwards past the valve gear, the piston, of course, being slightly lubricated to ensure a perfectly air-tight fit. The valve is also solid with the centre rod, H, terminating above in the knob, J. The pressure on the valve is applied through the crown of the piston via the case-hardened steel washer, K, to which in turn it may be applied either by the spring, L, acting through the ball thrust-race, M, for automatic control by a set tension in the spring, or by the long steel sleeve, NNN, which may

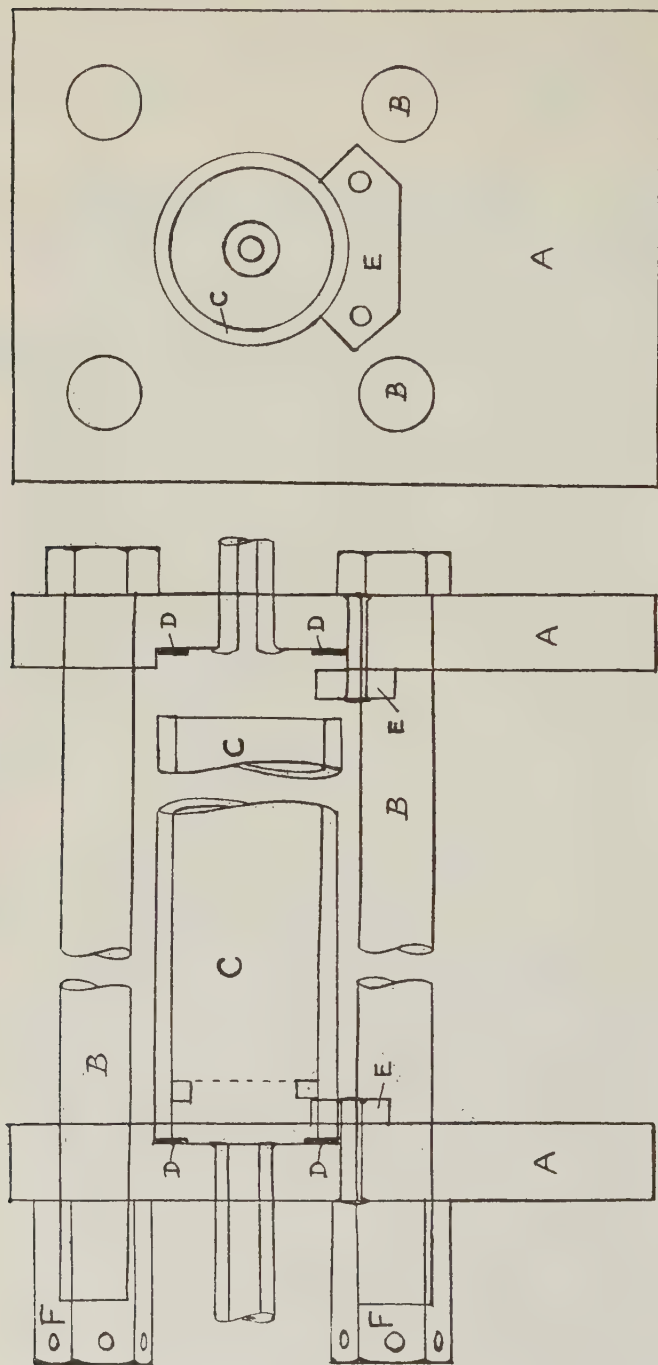


FIG. 2. Diagrams of steel drying tube (F in Fig. 1) for drying air under pressure. End view on right. For explanation see text, p. 631.

be screwed downwards through the large plug, o, by the milled nut, s, for direct control by hand. For automatic control the spring, L, is compressed to the required tension by screwing the plug, o, up or down in the block by rotating the milled head, p. When the correct setting has been found the

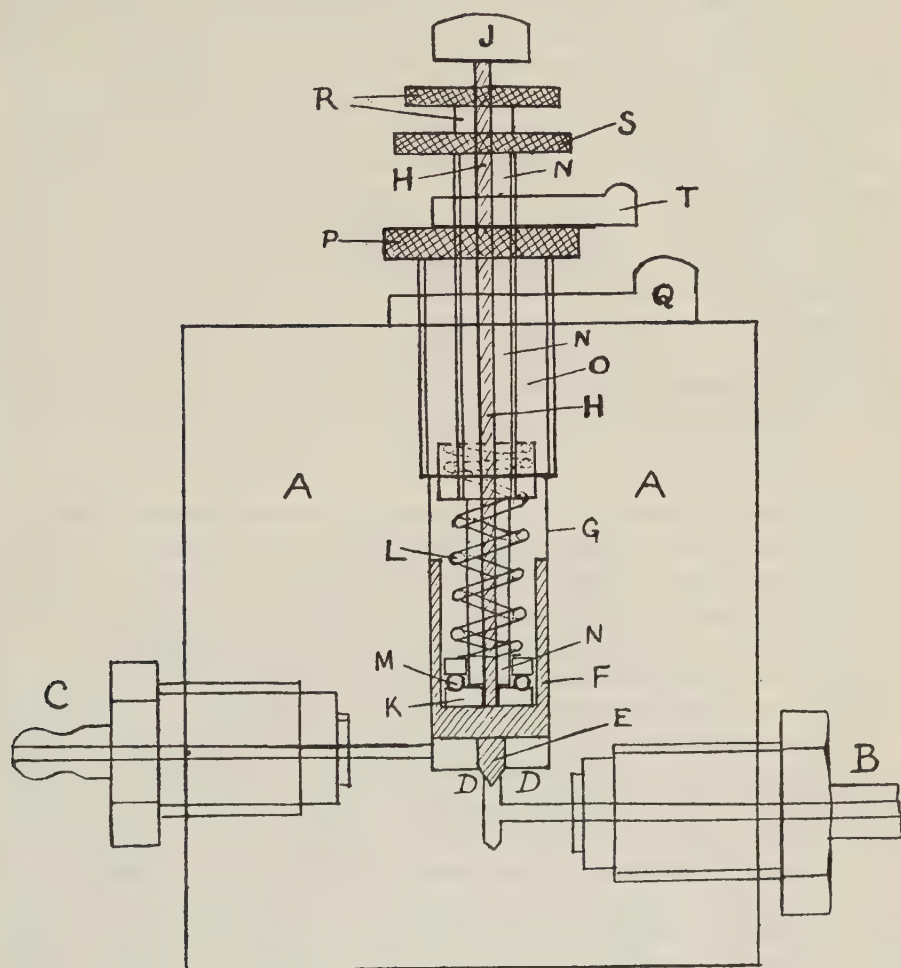


FIG. 3. Vertical sectional view of the outlet valve (o in Fig. 1). For explanation see text. p. 631.

plug, o, is locked in position by the lock-nut, q, which is fitted with a lateral lever for convenience in operation. The lower end of the plug, o, is recessed to receive the upper end of the spring. For accurate setting the spring and plug must turn together without any tendency to wind up or unwind the spring; hence the necessity for the ball-race at M. For manual control pressure is applied to the valve by screwing down the steel sleeve, nnn, by the milled head, s, the upper part of the sleeve being threaded through the plug, o,

which by tightening Q is locked solid to the block. It is also necessary, however, that an unscrewing motion of the milled head, s, should actively withdraw the valve and not merely leave it resting in its seat by its own weight, since the pressure required to lift it in this condition is very indefinite and varies, for instance, with the temperature and viscosity of the oil lubricating the piston. For this purpose when manual control is going to be used, R is first screwed down the central spindle, H, which can be held by the knob, J, to prevent it rotating until it is locked against the next milled head, s. This has the effect of drawing the valve and piston-crown and washer, K, up tightly against the end of the sleeve, NNN, so that the valve, centre spindle, and sleeve and milled heads, R and s, are all locked solidly together. The valve can then be accurately set or varied to a nicety in either direction by the milled head, s, alone. Since the spring necessarily remains under some tension, this operation also necessitates the presence of the thrust-rod, M, the spring not being free to turn. When it is required to maintain a definite constant setting, s may be locked in the required position by the lock-nut, T, which for convenience has a lateral lever extension and works against P in the same way as Q against the block.

Experimental method.

The details of procedure in an ordinary experiment are as follows. An experimental branch, previously allowed to attain saturation, is placed in the cylinder, the cylinder being closed with all the precautions already described (Haines, 1935, p. 224), and illuminated in the same way as in the earlier experiments (1935, p. 224). The drying tubes are charged with fresh calcium chloride, the tube, R (Fig. 1), is weighed together with the three-way tap, Q (Fig. 1), and reinserted in the circuit with the tap, Q, set in such a way that the tangential grooves in the solid key connect together the leads to the drying tube and simultaneously connect P directly with s. The outlet valve, O (Fig. 1), is opened wide by locking P by means of Q (Fig. 3), locking the sleeve, NNN, and centre spindle (as described above), and unscrewing the milled head, s, by several whole turns. Dry air is then passed through the apparatus at atmospheric pressure (ensured by the wide opening of the outlet valve) for one hour, it being shown by control experiments (cf. also Haines, 1935, p. 229) that the transpiration rate normally becomes constant (within 4 per cent.) under cylinder conditions in forty-five minutes. For this purpose the gas cylinder, D (Fig. 1), used as a reservoir, is pumped up from the compressor with the inlet valve at H closed, the valve, B, is closed and the compressor stopped. The flow of air through the apparatus is then started by opening the inlet valve, H, and regulated by this latter valve (H) alone to a speed of 0.45 litre per minute (see below, pp. 09-011), and maintained at this rate for one hour. To keep up the necessary pressure in D, the valve, B, may be opened and the compressor run from time to time as required.

At the end of this time the drying tube, R, is put into the circuit by the three-way tap, Q, and the flow at atmospheric pressure continued in the same way for a further hour. The three-way tap is then turned to close the drying tube, and the latter, together with the tap, is detached and reweighed to find the gain due to water vapour transpired during the hour at atmospheric pressure. The drying tube and tap are then reconnected, the tube being allowed to remain out of the circuit. The milled head, s, of the outlet valve (Fig. 3) is then screwed down to the point at which it only just allows the same rate of flow, and the pressure in the pressure cylinder is gradually raised by opening the inlet valve, H. The pressure is allowed to rise at a rate of approximately 2 atmospheres per minute until the desired pressure is attained. The outlet valve is then adjusted either directly by s (Fig. 3) or through the spring by P (according to the type of control it is proposed to use) to maintain the desired pressure in the cylinder, and the inlet valve, H, is adjusted to allow the correct rate of flow. The correct rate of flow is that which provides a scavenging effect equal to that at atmospheric pressure and will be discussed below (pp. 635-8). When the correct pressure and rate of flow have been attained the drying tube, R, is switched back into the circuit by the tap, Q (Fig. 1), and the water vapour transpired in one hour at the increased pressure is collected and determined in the same way as before.

The speed of the air current.

Inasmuch as the values actually required are the rates of evaporation under different pressures into still air, the speeds of the air current at all pressures are kept as low as is consistent with the removal of water at the same speed as it is evaporated, as low, that is, as is consistent with the prevention of any increase of humidity in the cylinder. To this end the minimum speed at which no accumulation of water vapour takes place in the cylinder at atmospheric pressure is found by preliminary experiments, and from this result are calculated the rates which should provide equal scavenging effects at the higher pressures. In this way a series of speeds of air current is obtained for use at the different pressures, at which the rates of removal of moisture by the streams are always equal to the rates of evaporation. At the same time the currents are of such slowness that the special effects of moving air scarcely come into play and a close approximation is obtained to the values for still air. Actually, the very slight air movement is bringing about the same reduction in vapour pressure in the outer layers of the diffusion systems in the confined space in the cylinder as would be allowed by the increased freedom for diffusion from these layers in the open. Suppose, for example, that a certain evaporating surface in the open loses water at such a rate that the vapour pressure at a point, say, 1 cm. from the surface is kept up to a value, p , in spite of the free space for diffusion. If the surface be then enclosed (e.g. a bell-jar placed over it), further diffusion from the outer parts of the diffusion system is prevented, so the vapour pressure at the given

distance of 1 cm. from the surface is increased, to say, p' . Evidently, if a slow current of air be passed through the enclosure or bell-jar, then at a certain speed of this current vapour will be removed at just such a rate that the vapour pressure at the given distance of 1 cm. from the surface will be restored to the original value, p , and the rate of removal of vapour by the air stream will be the same as the rate of spontaneous diffusion into open air. This would be the minimum speed for the particular surface and enclosure in question at which the rate at which vapour was carried over would be equivalent to the rate of evaporation into still air in the open, and it is the rate required in the experiments. At speeds of air current higher than this critical speed the rate of carrying over of vapour by the current would still be equal to the rate of evaporation, but the rate of evaporation would be artificially increased by the air movement and therefore greater than that in the open in still air. At all speeds higher than the critical speed required, the humidity in the enclosure must remain constant for any given speed and decrease with increasing speeds of flow. At speeds below the critical speed, on the other hand, the humidity within the enclosure will increase: this provides the means of determining the required critical speed, since with too slow a current the increase of humidity leads to an increase in the rate at which vapour is carried over with time at a constant rate of flow. A very slight increase in rate of flow, moreover, brings about a disproportionate increase in the rate of carrying over of water vapour, since accumulated vapour is carried over in addition to that which is newly evaporated at the higher speed. These points are easily detected by preliminary experiments and the critical speed therefore easily determined. These preliminary experiments were performed on free water surfaces, and in the later experiments on leafy branches leaf areas were used giving approximately the same total evaporation rate as the standard water surface. The rates found can therefore be used both for experiments with water surfaces and with branches. A glass dish 10 cm. in diameter and brim-full of water is placed under the collecting funnel in the cylinder and the rate at which vapour is carried over into the drying tube is followed up during successive periods at slightly different speeds of air current, the conditions being otherwise constant and the same as in the other experiments. With very slow air currents, e.g. 0.1 litre per minute, vapour is carried over at a very low speed, e.g. 0.1 gm. per hour, rising to 0.2 gm. per hour in the first two hours. Doubling the speed increases the value initially to 0.25 gm. per hour, but this rapidly falls off towards the original value. The same happens with all speeds (e.g. 0.18, 0.27, 0.35 litre per minute) up to 0.45 litre per minute. This appears to be the lowest speed at which there is no significant increase in the rate of collection of water vapour at constant speed, no pronounced effect of increase of speed (5 per cent. only as against 15 per cent. for a similar percentage increase in speed from the next lower speed used, 0.35 litre per minute), and no significant falling off from the new initial value after changing over to the next higher

speed. A speed of 0.45 litre per minute was therefore taken as correct for experiments at atmospheric pressure.

To equalize as nearly as possible the rates of flow in experiments with plants and with free water surfaces, the clearance of the collecting funnel from the brim of the glass dish, when such was used in control and preliminary experiments, is made such that the mean velocity of the stream over the water surface is approximately equal to the mean velocity of the air stream over the plant. The mean velocity over the plant is the speed of the air stream half-way up the funnel. The mean velocity over the water surface, the velocity in the centre being zero, may be taken as roughly half that at the edges. The velocity at the edges of the dish must, therefore, be double the velocity half-way up the funnel. Since the mouth of the funnel measures 10 cm. in diameter, the mean velocity of the air passing over the plant under the funnel (at 0.45 litre per minute) is $450/(3.14 \times 2.5^2)$ cm. per minute, or approximately 23 cm. per minute. The clearance between the edges of the funnel and the brim of the dish is therefore made roughly 3 mm., so that the area of the annular opening admitting air to the funnel ($10 \times 3.14 \times 0.3$ sq. cm.) shall be approximately half that of the mean section of the funnel (3.14×2.5^2 sq. cm.). Thus the mean velocity of the current over the water surface taken as half its velocity at the periphery becomes equal to the mean velocity in the funnel. The critical velocity of air current of 0.45 litre per minute, found as described above, gives a mean speed in the funnel over the leaf surfaces of 23 cm. per minute, or 0.38 cm. per second at atmospheric pressure.

At other pressures it is evident that equal scavenging effects will be produced by an equal speed of air movement over the plant. This would mean an increased volume of air per minute (measured at atmospheric pressure) in proportion to the increased pressure. Thus, when the pressure is increased from zero to 1 atmosphere the actual absolute pressure is increased from 1 atmosphere to 2 atmospheres, and twice the volume of air (measured at atmospheric pressure) must be passed through the apparatus per unit time to provide equally efficient flushing out of water vapour. At 2 atmospheres deficit (three times the original absolute pressure) the rate would require to be three times the original rate, and so on. It might be argued that as the pressure increases the rate of evaporation is so much reduced thereby—as shown both by the present and all the earlier series of experiments—that at the higher pressures only a smaller and smaller volume of air measured at the relevant pressures (i.e. a slower and slower current) will suffice to carry over the water vapour at the same rate as it is transpired. A little consideration will show, however, that such a reduction in the rate of flow would allow an artificially increased vapour pressure near the leaf surfaces at the higher pressures as compared with the lower, and the evaporation at the higher pressures would therefore appear too low. The normal gradient-steepening effect of pressure would be reduced. In still air, an increase of pressure reduces the evaporation rate and therefore reduces the humidity at a given

distance from the surfaces. Thus at higher pressures the vapour-pressure gradient near the evaporating surfaces is steepened as a result of the reduced mean free path of the molecules and consequent reduced diffusion rate of the water vapour. Steepening of the gradient or reduction of the vapour pressure at a small distance from the surface tends to increase the rate of evaporation. The effect of pressure is therefore not as great as it would be if this secondary effect of steepening of the gradient did not come into play. This effect would be reduced by reducing the speed of the air current. It may be noted in passing that in moving air this secondary effect is of scarcely any account since the gradient is already so steep, even at atmospheric pressure, that the further effect of pressure is almost negligible. It therefore follows that the effect of increased pressure will not be as great in still air as in moving air. The value required is the closest possible approximation to the value for still air, and it is therefore obviously not desirable to reduce the rates of flow at higher pressures in consideration of the reduced evaporation rates, or the values obtained for actual evaporation will be too low. Such a reduction would, in fact, introduce some compensation for the very effect it is required to measure. The rates of flow are therefore increased in proportion to the actual pressures.

The relative rates used at different pressure deficits and their equivalents in linear speeds of movement over the evaporating surfaces and in numbers of changes per hour of the total air in the cylinder and the collecting funnel are given in Table I. They are obtained during experiments by timing the

TABLE I

Deficit (lb./in.)	Actual pressure in atmospheres.	Rates of flow of air			No. of changes of air per hr.	
		Litres per min.	Cm./sec. at surfaces.	Litres per hr.	Cylinder.	Funnel.
0	1 = 1	0.45	0.38	27	16.5	289
50	1 + 3.33 = 4.33	1.95	0.38	117	71	1,240
100	1 + 6.66 = 7.66	3.45	0.38	207	126	2,200
150	1 + 10 = 11	4.95	0.38	297	181	3,160
200	1 + 13.33 = 14.33	6.45	0.38	387	236	4,130

$$\text{Volume of cylinder} = \pi r^2 h = 3.14 \times 9 \times 9 \text{ cu. in.} = 254 \text{ cu. in.} = 1,640 \text{ c.c.}$$

$$\text{Volume of collecting funnel} = \pi r^2 h/3 = 3.14 \times 4 \times 2\sqrt{3}/3 \text{ cu. in.} \\ = 14.5 \text{ cu. in.} = 94 \text{ c.c.}$$

$$\text{Volume of funnel} = \text{volume of cylinder} \times 1/17.5.$$

$$0.45 \text{ litre/min.} = 27 \text{ litres/hour} = 27,000/2.54^2 \text{ cu. in./hr.} = 4,185 \text{ cu. in./hr.} \\ = 4,185/254 \text{ cylinder volumes/hour} = 16.47 \text{ changes/hour} \\ (= 27,000/1,640 \text{ cylinder volumes/hour} = 16.47 \text{ changes/hour})$$

at atmospheric pressure.

rates of bubbling through the bottle of sulphuric acid at τ (Fig. 1) with a stop-watch and calibrating these rates from time to time by collecting the outcoming air in gas cylinders and directly measuring the volumes issuing per unit time.

It will be realized from the table that since the volume of air passing per hour through the apparatus amounts to from 289 to 4,130 times the volume

of the collecting funnel containing the evaporating surfaces, the flushing is amply sufficient, although the speed of the current over the evaporating surfaces is too slow to bring about any appreciable effects of air movement. In any case, any such effects could not vitiate the required ratio of the transpiration rates under different pressures, since, owing to the adjustment of the rates of the air stream, they become similar for all pressures.

TABLE II

Pressures (lb./in.):	0	50	100	150	200
a. Experimental values for free water surface: (in gm. water evaporated per hour)	0.346				
	0.364	0.072	0.039	0.028	0.020
	0.363	0.075	0.038	0.027	0.022
	0.379	0.072	0.039	0.030	0.021
	0.347				
Means:	0.360	0.073	0.039	0.028	0.021
Percentage values:	100	20	11	8	6
b. Theoretical values for free water surface:	100	23	13	9	7
c. Experimental values for Aesculus with different pressures at leaf surfaces, but zero deficit, all as percentages of rate at atmospheric pressure:	100	37	17.5	11	
	100	31	16	9	6
	100	30	14.5	8.5	5
	100	24	11.5	7.5	
Means:	100	30.5	15	9	5.5
d. Experimental values for Aesculus with different pressures at leaf surfaces and corresponding deficits, the tracts being throughout at atmospheric pressure. All values as percentages of rates at atmospheric pressure.	100	47.5	23		
	100	47	22.5	21	21
	100	46	20	19	20
	100	41	19.5	19	16.5
	100	40	19	19	16.5
	100	38	19	14.5	15
	100	36.5	18.5	13.5	12
	100	35.5	18.5	11	11
	100	33.5	16.5	10	9
	100	32.5	15.5	9	7
	100	31.5	14.5	8	6
	100	31	14.5		
Means:	100	38	18	14.5	13

Types of experiment performed.

Three types of experiment have been carried out by the method described: (a) Normal experiments on Aesculus branches in which the butt end of the branch was passed through the base of the cylinder in the usual way so that increase of pressure in the cylinder led to increased pressure at the leaf surfaces and also to a pressure deficit. (b) Control experiments on branches of Aesculus in which shorter branches were used, the base of the cylinder being sealed and the butt end of the branch being allowed to dip into a small vessel of water enclosed within the cylinder with a layer of oil on the water surface to prevent evaporation. In these experiments there is increased pressure at the leaf surfaces but no deficit. (c) Control experiments on free water surfaces, in which the base of the cylinder was sealed as in (b) and a flat dish of equal diameter to that of the mouth of the collecting funnel

and brim-full of water was placed in the cylinder, 0.3 cm. below the rim of the funnel. These experiments show the effect of increased pressure at the evaporating surface on the rate of simple evaporation.

In both the types of experiment (*a*) and (*b*), in which leafy branches were used, the values for the higher pressures were determined shortly after the values for the next lower pressure, without reduction of the pressure in the interim. In this way the periods during which the cell sizes are not yet characteristic of the equilibrium at the newly reached pressure are shortened and errors due to changes of transpiration rate resulting merely from contraction of the cells (Haines, 1935*a*, p. 559 et seq., Fig. 6) are minimized. The readings are therefore taken during successive hourly periods (with short intervals between for weighings and for increase to the next pressure) at 0, 50, 100, 150, and 200 lb./in. respectively, and always only during such times as the leaf-cell sizes and evaporation rates have come to equilibrium at the relevant pressures. The experiments on water surfaces were conducted on similar lines.

RESULTS AND DISCUSSION

The results of all experiments are summarized in Table II and plotted in Figs. 4 and 5. In the case of the free water surface (Table II, *a*) it will be seen that, as would be expected on theoretical grounds, the evaporation rate decreases roughly in proportion to the absolute pressure. Thus, at an applied pressure of 1 atmosphere the actual pressure becomes 2 atmospheres, or double that when no excess pressure is applied and the rate is halved. At an excess pressure of 3 atmospheres the actual pressure becomes 4 atmospheres, or four times that when no pressure is applied and the rate is reduced to a quarter and so on. The theoretical figures worked out on this basis are given for comparison in Table II, *b* and plotted in Fig. 5 (curve *b*) side by side with the experimental curve (curve *a*) obtained by plotting the figures in Table II, *a*. The curves may be considered to be in good agreement. Actually, the experimental curve lies slightly below the theoretical curve, but it is doubtful whether the difference is significant.

In the experiments on leafy branches under pressure, but kept at zero pressure deficit by enclosing the base of the branch and water supply also within the cylinder, the evaporation rate is reduced by pressure in much the same way as for a water surface. The results are given in Table II, *c* and the individual points are plotted to show the nature of the error in the upper graph of Fig. 4. The curve of nearest approach is drawn through these points in Fig. 4 and also given in Fig. 5 (curve *c*) for comparison with the other curves. It would appear that the plant is somewhat less affected by pressure than the free water surface, especially at the lower pressures, a result which was not expected and which is without any obvious explanation. It would be expected that any direct effect of pressure on the stomata would make for closure and lead to the opposite result, but this is manifestly a point for further investigation. Seeing that the effect of pressure is due to reduction

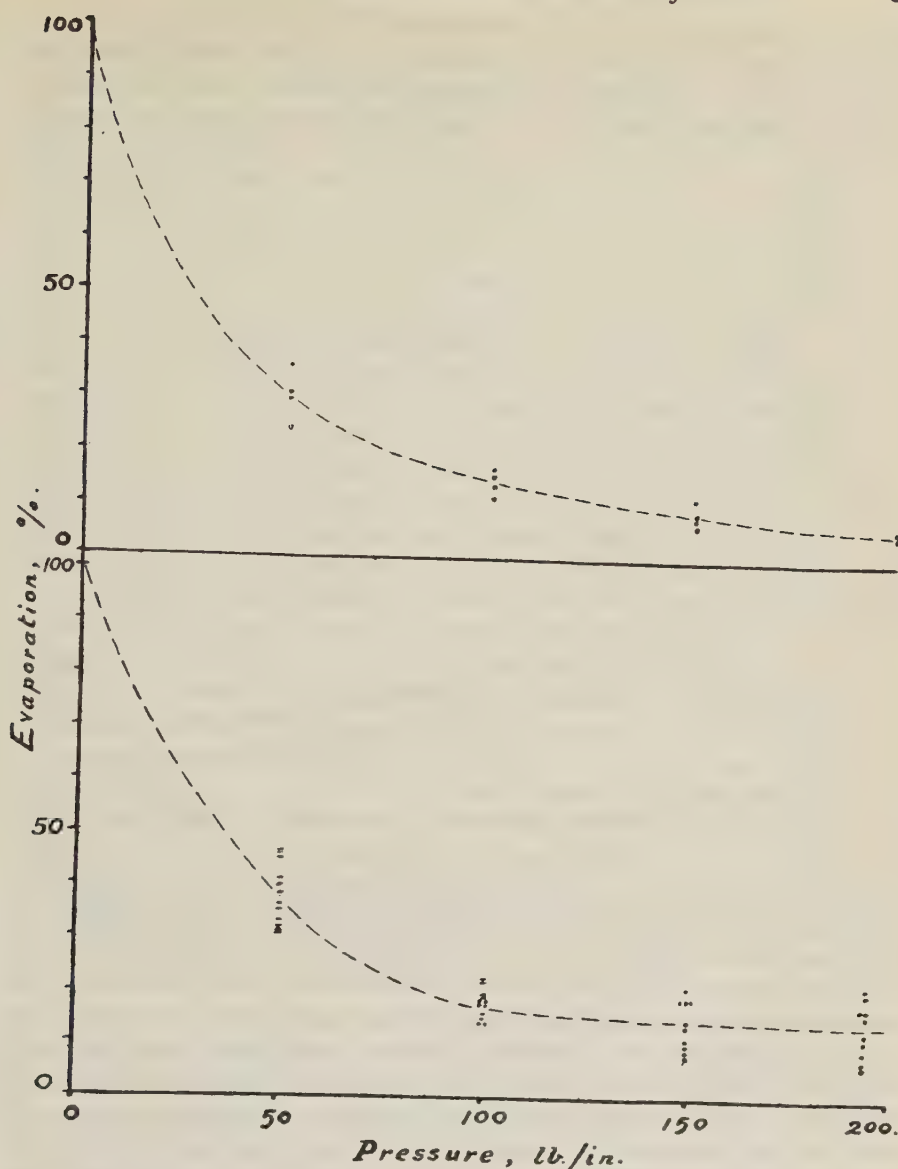


FIG. 4. Graphs of the mean values given in Table II. Upper curve from *c* of Table II, for plants under pressure at leaf surfaces but without pressure deficit. Lower curve from *d* of Table II, for plants under pressure at leaf surfaces and with corresponding pressure deficits.

of mean free path of diffusing molecules after they have escaped from the evaporating surface, it might appear at first sight to be of the nature of a physical impossibility that under increased pressure transpiration should become relatively faster than evaporation from a water surface. This, however,

is not the case, since it has to be remembered that the transpiration per unit area of leaf surface is only approximately one-half the evaporation from an equal area of water surface at atmospheric pressure. The difference is due to the fact that in the leaf the true evaporating surfaces are enclosed

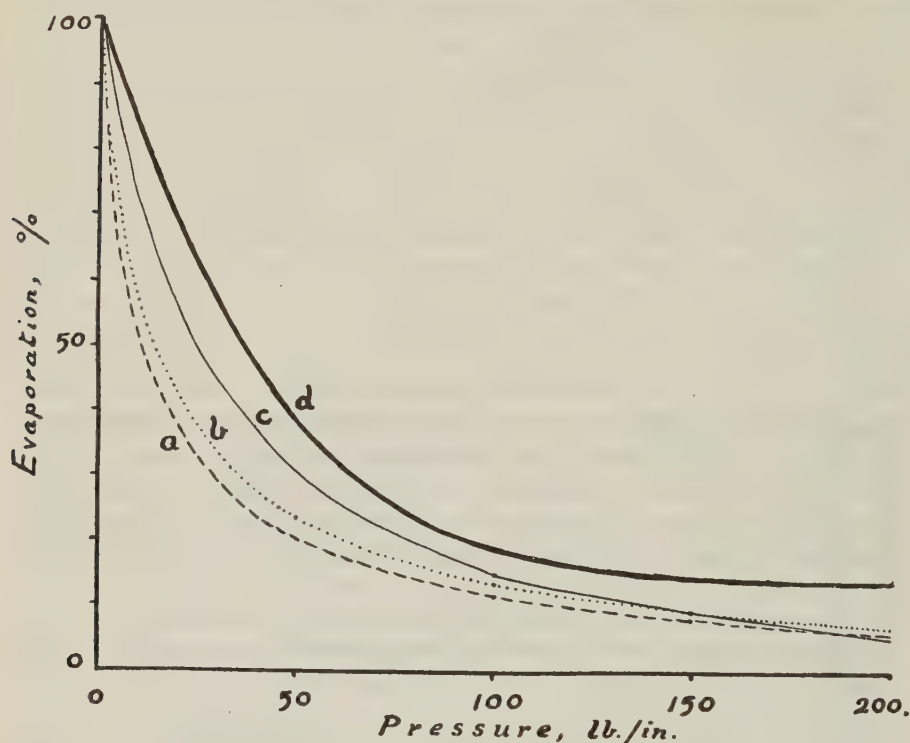


FIG. 5. Graphs of the mean values given in Table II. Curve *a* (from *a* of Table II), experimental curve for free water surface. Curve *b* (from *b* of Table II), theoretical curve for free water surface. Curve *c* (from *c* of Table II), plants with no deficits. Curve *d* (from *d* of Table II), plants with deficits. For further explanation see text, p. 640 *et seq.*

behind the stomatal system and that diffusing vapour, before reaching the latter, must first penetrate a network of intercellular spaces in the leaf. Although the effects of pressure due to reduction of diffusion rate in the surrounding atmosphere must necessarily be the same for the leaf and for the water surface, two possible mechanisms suggest themselves whereby the points of structure peculiar to the leaf may cause it to be relatively less affected by pressure than the free water surface. Firstly, if it be supposed that the stomatal spacing be slightly too crowded for maximum efficiency at atmospheric pressure, under normal conditions the diffusion systems will slightly interfere, leading to loss of efficiency and bringing down the normal transpiration rate as compared with its possible theoretical value with spacing of maximum efficiency. Under increased pressure the vapour-pressure gradient outside each stomatal aperture is steepened as a result of the reduced

rate of diffusion, and there will in consequence be less interference between adjacent diffusion systems. A slight increase in efficiency of the stomatal system results, and the transpiration rate is therefore not lowered quite as much as would otherwise be expected and not reduced as much (relative to its original rate) as the evaporation from a free water surface. The second possible explanation is that under pressure the cells of the mesophyll of the leaf become reduced in size with a more or less corresponding increase in the sizes of the intercellular spaces. The whole structure cannot contract together, at least in some directions, on account of the supporting action of the more rigid structures such as the cuticle and vascular bundles. This will lead to increased efficiency for diffusion of the internal system of the leaf, and again the transpiration under pressure will not be reduced in the same proportion as the evaporation from a free water surface. These suggestions are obviously only conjectural, but serve to show that the sequence of the graphs in Fig. 5, though unexpected, is not a physical impossibility.

By far the most unexpected result is the relation between the above discussed graph for leafy branches at zero deficit and that for branches under deficits, obtained by experiments similar to those of the earlier series with the butts passing out through the base of the cylinder into a water supply at atmospheric pressure. The results of these experiments are given in Table II, *d*, and the individual points plotted to show the error on the lower graph in Fig. 4. The curve of nearest approach is drawn through these points in Fig. 4 and also given by curve *d* in Fig. 5. The somewhat surprising conclusion is reached that increased pressure at the leaf surfaces combined with a deficit does not reduce the transpiration as much as the increased pressure at the leaf surfaces alone. In other words, at least under the experimental conditions of generally increased pressure, a pressure deficit, which has always been assumed to reduce transpiration, far from causing any substantial reduction in transpiration tends if anything to allow an increase. It will be realized from Fig. 4 that the experimental errors where leaf surfaces are concerned are still rather large, evidently on account of a lack of physiological uniformity of the material, since the results for water surfaces are very much more consistent. In view of this it cannot be said that the evidence for an actual increase in transpiration as a result of a deficit is as yet quite conclusive, but it is at any rate evident that a deficit *per se* (at least at the range of actual pressures used in the experiments) does not cause any appreciable reduction in transpiration rate. This is contrary to expectations. A pressure deficit of 200 lb./in. would be expected to reduce the water content to 75 per cent. even of that at 10 atmospheres (150 lb./in.) deficit and therefore to reduce the water content at turgidity by considerably more than 25 per cent. This in view of existing work on the relation between transpiration rate and leaf water content (Knight, 1917; Livingston and Brown, 1912) would be expected to lead to a substantially reduced rate of transpiration, though no theoretical reason for the reductions observed by these writers can be suggested.

The extent of the reduction expected theoretically will be clear from the following considerations. A fully turgid mesophyll cell would be expected to be in equilibrium with a fully imbibed cell-wall. The vapour pressure at its surface should therefore approximate to that of a free water surface at the same temperature. (Actually it will be slightly lower, since the extra-cellular liquid cannot be pure water.) When a pressure deficit of, say, 10 atmospheres is applied, the cell would be expected to lose water to the conducting tracts until its suction pressure became 10 atmospheres. Since this is the approximate point (for *Aesculus*) at which reversal of flow occurs (i.e. the point at which all natural turgor pressure is removed and the applied pressure becomes equal to the osmotic pressure of the sap), it may be taken that the state of imbibition of the cell-wall will now be such as to bring it into equilibrium with a cell-sap of ten atmospheres osmotic pressure. The vapour pressure at its surface should therefore have fallen approximately in the ratio of the vapour pressure of a 10-atmosphere solution to the vapour pressure of water at the same temperature, and the transpiration should have fallen to the same extent. Reckoning cell solutes as potassium nitrate and taking the temperature as 15° C., the ratio becomes 12.691:12.788, and the transpiration should fall to 99.1 per cent. of the original value. In practice, however, it is evident that no such relation exists. In view of the magnitude of the errors, the present experiments do not completely rule out the possibility of such a relation holding under experimental conditions, since a reduction of transpiration in exact correspondence with the reduced vapour pressure could not be detected. The apparently significant rise obtained, however, makes it appear very unlikely. On the other hand, it is certainly not true in nature. Livingston and Brown (1912) observed a range of transpiration rates considerably greater than that which could be accounted for by the changes in vapour pressure. Fitting (1911) and Livingston (1911) find the osmotic pressure of the cell-sap in the leaves of desert plants in moist soil is approximately equivalent to that of 2M potassium nitrate. Livingston and Brown (1912) therefore argue that the maximum possible range of cell-sap concentration in these plants is from 3M to zero. This is undoubtedly a liberal overestimate, since there is no evidence that the value ever rises appreciably above 2M on the one hand and it cannot possibly approach very near to zero on the other. Even so, if the range of transpiration rates were proportional to the vapour pressures at the cell surfaces, and these in turn were proportional to the osmotic concentrations of the sap, as might reasonably be expected, the maximum change in transpiration rate (ignoring stomatal effects) would amount only to a reduction of 8 per cent. The observed value of 49 per cent. (Livingston and Brown, 1912) cannot therefore be explained as a result of the change in sap concentration and vapour pressure alone. Livingston and Brown suggest that the reduction in transpiration rate may also be due in part to other factors such as increased concentration of the extra-cellular liquid, increased concentration of the cell-sap by added solutes

(e.g. by photosynthesis), or a decrease in the permeability of the protoplast. A similar suggestion to this last has been made by Haines (1935*a*), but in view of the present results and the consequent changes in the situation it cannot now be said until further data have been obtained whether this is a profitable hypothesis or not. (It depends upon whether the lack of agreement with theory lies in the relation of deficit to water content, of water content to superficial vapour pressure, or vapour pressure to transpiration rate—and this is not yet known.) Whatever may be the mechanism by which it is brought about, all the work, however, indicates a marked reduction in transpiration rate with falling leaf water content. Knight (1917) came to the same conclusion. In one of his experiments the values for transpiration rate range from 7,200 units to 2,860: a reduction to less than 40 per cent. of the higher value. The changes are ascribed to changes in leaf water content, though the corresponding leaf water contents are not given. The suggestion that the reduction depends upon a reduction in leaf water content is probably true, but leaf water content in that case evidently does not work only through the resulting decrease in vapour pressure at the cell surfaces. Either the vapour pressure at the cell surfaces fails to bear the expected relation to cell-sap concentration and therefore to water content, or this relation is normal, but the transpiration rate mainly determined by some other conditions also dependent upon water content. Whatever the mechanism, transpiration is evidently markedly reduced in nature even by relatively small decreases in leaf water content, and it is difficult to see how the latter, and therefore transpiration also, can escape reduction in pressure deficit experiments.

The position is therefore as follows. Falling leaf water content is known to be accompanied by a considerable reduction in transpiration rate, which may be to 40 per cent. or less of the original value. This might be supposed to be due to falling vapour pressure at the cell surfaces due to a reduced state of imbibition accompanying the drying out of the cells. This, however, can only account for a reduction in transpiration rate of 8 per cent. at the very most, and normally only approximately 1 per cent. as against 60 per cent. or more observed. The reduction in vapour pressure at the cell surfaces and drying out of the cells would be expected to be proportional to the pressure deficit when such was applied, and transpiration rate would therefore be expected to fall (within a relatively small range at least) in response to pressure deficit. Inasmuch as drying out in nature leads to a markedly reduced transpiration rate, it would also be expected that the drying out which must presumably result from an experimentally applied deficit would also lead to a marked reduction in transpiration. This could not operate through reduced vapour pressure at the cell surfaces alone but would be expected to operate by the same mechanism, whatever it may be, as the naturally produced deficit. Applied pressure deficits, however, do not apparently reduce transpiration to a measurable extent at all, but probably even

increase it. The experimental errors are at present too large to enable it to be stated decisively that a large experimentally applied deficit does not cause a small decrease in transpiration corresponding with the reduced vapour pressure. The evidence suggests, however, that even such a reduction is very unlikely and that considerable reductions such as would be expected from the extent to which transpiration falls off with water content in nature are out of the question. It is therefore certain on the one hand that in nature a falling leaf water content must operate in some other and more complex way than through the vapour pressures at the cell surfaces alone, and probable on the other that the vapour pressure at the cell surfaces does not bear the expected relation to the pressure deficit or presumably, therefore, to the concentration of the cell-sap. It is also possible, however, that the expected relations between the pressure deficit, cell-sap concentration, and vapour pressure do exist, but that the rate of transpiration is principally decided by some other condition altogether, compared with the effects of which those due to alterations in vapour pressure are very small and therefore not observed in practice. A further possibility is that water content may bear the expected relation to pressure deficit in nature, but that the relation is altered in some way in the experiments, though it is difficult to see how, by shifting all the pressure values up the scale. Direct information is evidently desirable on the relation between pressure deficit and leaf water content. A still further possibility is that the normal effect of the deficit on the leaf water content may not be reached in the experiments owing to the deficit being artificially increased at too great a rate as compared with that at which similar deficits are attained in nature. This seems unlikely. On the other hand, it has to be remembered that in nature the normally observed falling leaf water content (for instance towards midday in the daily cycle; cf. Livingston, 1912) is the cause and not the result of the deficit as it is in the experiments. The effect in both cases may be expected to lag behind the cause. The leaf water content corresponding with a given deficit in nature may therefore be far below that corresponding to a similar deficit applied experimentally. In nature the deficit shows the lag, and in the experiments the leaf water content. The transpiration rate in the experiments will not therefore be as low as in nature. Special experiments on the effects of small deficits (Haines, 1936a, and Humphries, 1938) have shown that these undoubtedly cause an increase in transpiration rate. The apparent increase in transpiration by larger deficits may therefore also be true and linked up with the same mechanism. In this case the reduction in transpiration rate with falling leaf water content in nature may be due to the falling leaf water content without bearing the expected relation to the pressure in the tracts. It is at least evident that the general problem of the relations between transpiration rate, leaf water content, and pressure deficit is considerably more complicated than has been supposed. A simple reduction in transpiration rate in accordance with the reduced vapour pressure at the cell surfaces alone (assuming this to fall in

relation to the drying out of the wall which is in equilibrium with the increasing osmotic pressure of the cell contents as the cell diminishes in volume) is far from adequate to account for the observed reductions in transpiration rate.

The position is best summed up by summarizing the theoretical expectations and the stages at which they are known not to be fulfilled in practice. If the plant were a purely physical system, it would be expected that the transpiration rate would be proportional to the vapour pressure at the cell surfaces. This should be proportional to the water content of the cell and inversely proportional to the osmotic pressure of the cell contents. The osmotic pressure of the sap should be proportional to the deficit, and transpiration rate should therefore be inversely proportional to the deficit. In practice transpiration does not bear the expected relation to the deficit, as it is not reduced by increased deficit as much as would be expected and does not bear the expected relation to water content, since with falling water content it becomes reduced too much. If the transpiration rate is decided more by other unknown factors than by the vapour pressure, the theoretical relation may yet hold good between deficit, osmotic concentration, water content, and vapour pressure. On the other hand, further at present obscure factors may come into play to prevent these relations holding at any stage. These might be due to changes in the colloidal condition of some of the cell constituents, rendering a purely osmotic interpretation of the cell behaviour altogether inadequate. A detailed discussion of the possible nature of such factors, however, is obviously not profitable until more data have been obtained.

SUMMARY

The relation between transpiration rate and pressure deficit has been investigated by weighing the water vapour transpired from leafy branches of *Aesculus* placed under different pressures in a pressure cylinder. A special arrangement of valves allowed dry air to be passed over the plant under pressure and the transpired water to be collected and weighed at atmospheric pressure in the usual way.

Similar experiments have been conducted with free water surfaces and with plants with the leaves under increased pressure but with no deficit, the pressure in the tracts being similarly increased.

Transpiration appears, if anything, to be slightly less affected by pressure than evaporation from a free water surface. Reasons for this are suggested.

A pressure deficit in addition to increased pressure at the leaf surfaces reduces transpiration no more than increased pressure at the leaf surfaces alone. A deficit, at least in the range of actual pressures used in the experiments, therefore appears to have no marked effect upon transpiration rate. It certainly causes no marked reduction but may cause a slight increase, as has already been found to occur with small deficits. It is pointed out that

the exact cause of the reduction of transpiration in nature with falling leaf water content and the general relations between transpiration rate, leaf water content, vapour pressure at the cell surfaces, and pressure deficit in nature are therefore largely obscure. Further data are required.

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Studies in Flower Structure

IV. On the Gynaeceum of Papaver and Related Genera¹

BY

AGNES ARBER

With seven Figures in the Text

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I. INTRODUCTION

THE morphological interpretation of the gynaeceum in the Papaveraceae has been a matter for discussion since the time of A. P. de Candolle (1827), and widely varying opinions on the subject have been expressed by botanists of the present day. In the hope of obtaining further evidence bearing on the subject I began, some years ago, to cut serial sections of the flowers of members of this family; and, after examining a number of genera, I came to the conclusion that a special study of the genus *Papaver* might be a useful contribution. This genus was chosen partly because it affords a good test case, and partly because—although it is so familiar an object—our knowledge of its construction is still imperfect. Lignier's pioneer work (1911, 1915) on the anatomy of the gynaeceum in the Papaveraceae, which was left unfinished at his death, did not include *Papaver*, and later writers who have considered the genus have given accounts which are incomplete in certain critical directions.

In the following pages my observations on *Papaver* and related genera will be described, and their theoretical bearing will then be discussed. The terminology used in the descriptions and figures will be based, provisionally, upon the hypothesis that the carpels form one whorl of similar members, and that the placentae are joint outgrowths from their united margins. This is the theory which, on the evidence of external features, has been adopted generally by systematists, but it has never been tested by detailed application to the minuter structure of the gynaeceum of *Papaver*. When this gynaeceum

¹ This paper represents part of the work carried out during the tenure of a Leverhulme Research Fellowship. The writer is indebted for material to the Director of the Cambridge Botanic Garden.

has been described in full on this basis, we shall be in a position to determine whether, from the point of view of structural evidence, the theory is self-consistent, and also whether it enables us to relate the gynaecium of *Papaver* to that of other members of the family.

II. OBSERVATIONS

It is convenient to begin the study of *Papaver* with a species which has relatively few carpels, and, for this reason, *P. Argemone* L. may be used as a type. The fruit is seen in Fig. 1, A, while B1–B6 represent transverse sections from a series through a very young gynaecium. In each of these sections one of the carpels is, for clearness, delimited by arrows. B1, cut at the extreme base, shows the four carpels surrounding a minute central area of tissue, differing from them histologically; this is the vestigial apex of the floral axis. Each carpel at this level has its placental outgrowths appressed to enclose a loculus. The median strands of the carpels are scarcely distinguishable. There is no lignification except in the bundles on the radii of the placentae; these strands are interpreted as the united marginals of adjacent carpels. B2 is cut above the tip of the floral axis. The sterile basal regions of the four placentae now project freely into the cavity, and the median bundles of the carpels are becoming better defined than at lower levels. In B3 we pass to the ovule-bearing region of the ovary. The gynaecium is so young that the ovules are still atropous, though the rudiments of the integuments are already visible. This diagram may be compared with Fig. 2, C1, which represents the fertile region of an older gynaecium of the six-carpelled type. In Fig. 1, B4, we reach the level of the upper sterile region of the ovary, where it is passing into the stigmatic crown. We see here the reappearance of the individual loculi (cf. also Fig. 2, C3a), which were defined at the base of the ovary (B1), but which, between these levels, were merged in the general cavity. In B5, which is still higher, not only the loculi, but also the median regions of the carpels, are dying out, and they have disappeared before B6 is reached. In B4 and B5, as well as in Fig. 2, C3a and C4a, the centre of the section is occupied by a transmitting canal lined with papillae. In B4 the transmitting canal has four rays—the slits between the appressed placentae. In B5, and in Fig. 2, C3a, a second set of rays has developed, alternating with the first. These secondary rays indicate the beginning of the division of each placenta along its median plane; this bisection separates the half-placentae belonging to adjacent carpels, which, up to this level, have been united. It is the faces parted by this cleavage which bear the receptive papillae (Fig. 2, C5 and C6); they form the paired stigmatic rays of which an external view is given in Fig. 2, D.

In correlation with the splitting of the placenta, the fused marginals divide—each half passing to the carpel to which it belongs. This change takes place between Fig. 1, B5 and B6, but the vascular tissue is so embryonic in this series of sections that the process is difficult to follow. It can be

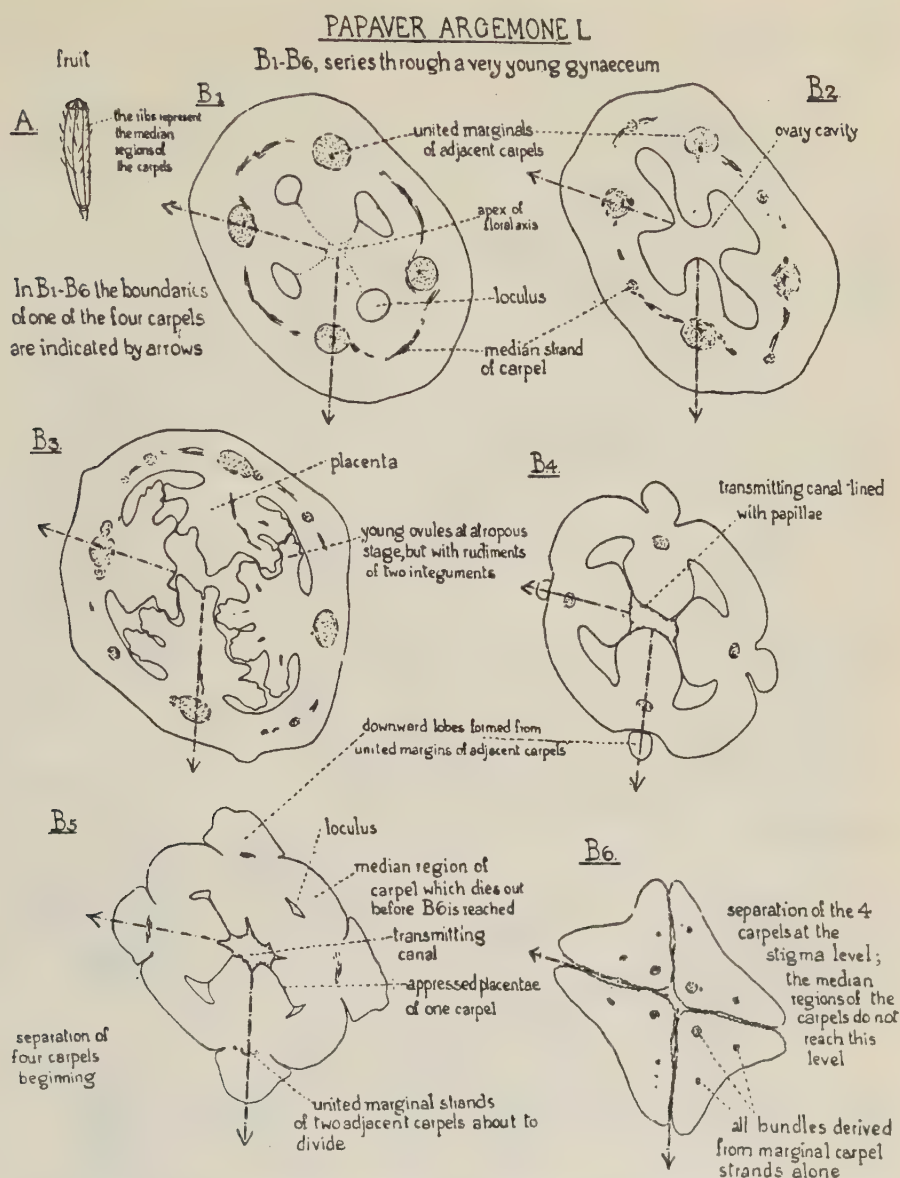


FIG. 1. (The orientation of the gynaecium in relation to the rest of the flower is not indicated in any of the figures in this paper. All the series of sections are traced from below upwards.) *Papaver Argemone* L. A, fruit ($\times 1$ circa). B1-B6, sections from a transverse series through a very young gynaecium, Histon, 27 May 1930 ($\times 47$). In each diagram arrows define the margins of one of the four carpels. B1, extreme base of gynaecium; B6, the four stigmatic members.

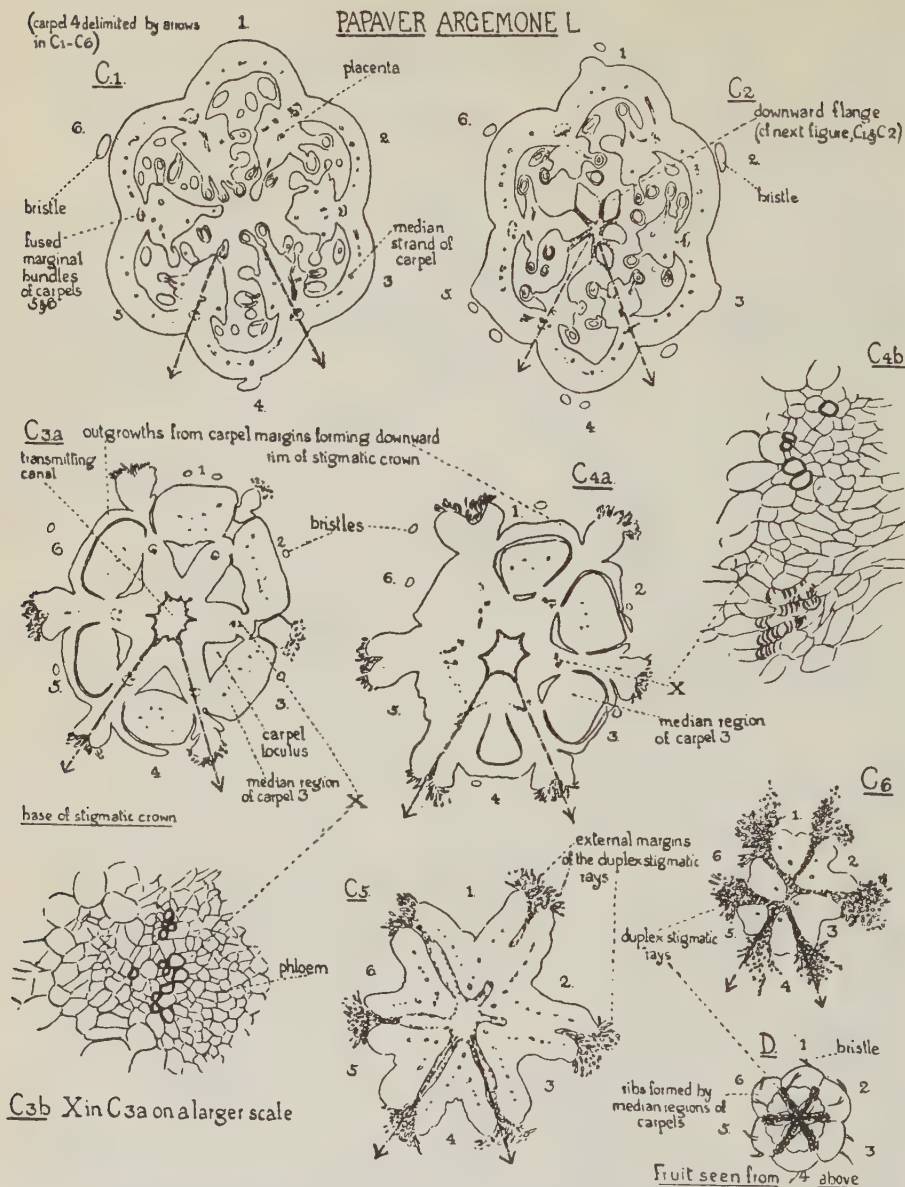


FIG. 2. *Papaver Argemone* L. C1-C6, sections from a transverse series through the gynaecium of an unopened bud, Camb. Bot. Gard., 25 June 1936 ($\times 14$, except C3b and C4b, which are $\times 193$ circa). For convenience of description the conventional numbering, 1-6, is used for the carpels, C1, to the stigmatic members, C6. D, fruit seen from above as a solid object (enlarged).

traced in detail, however, in the series through the older gynaecium drawn in Fig. 2. The upper sterile regions of the placentae in the base of the stigmatic crown are seen in Fig. 2, c3a; the bundles to the right of the diagram each show one xylem group, while in two of those to the left (which, owing to a slight obliquity in the section, are cut at a higher level) division is taking place. One of the individual bundles, x, is drawn on a larger scale in c3b. In c4a, which is a little higher in the gynaecium, division is proceeding, and the result is seen in detail for bundle x in c4b; the xylem now forms two separate groups. The completion of these divisions represents the entire disengagement of the vascular system of each carpel from that of its neighbours, and in Fig. 1, B6, and 2, c5, each of the terminal members is seen to possess its own arc.

We have now traced the derivation of these stigmatic members, and their vascular supply, each from a single carpel; in Fig. 2, D, they are seen in external view, each standing above the median region of the carpel to which it belongs. They do not, however, each represent a whole carpel, but they are formed exclusively from its margins and placental outgrowths, which extend above, and arch over, the reduced median regions. The disappearance of the median regions of the carpels, and of the loculi, as the stigmatic crown was reached, was observed in outline in the young gynaecium of the series in Fig. 1, B, but for a better demonstration we must go to the older series in Fig. 2. The condition in the main part of the ovary can be seen in Fig. 2, c1 and c2. In c3 we are reaching the base of the stigmatic crown, and the median regions of the carpels are reducing in width, but increasing in depth from back to front. The bundles are now scattered instead of forming a single series; in c4a, in carpels 1 and 2 the number of bundles is reduced, and in carpels 3 and 4 their upper limit is passed, so that there are no longer any to be seen. In this diagram, in carpels 1, 2, and 3, which are cut at a lower level than the rest, the attachments of the median regions to the placentae are narrowing, and withdrawing towards the centre of the gynaecium, thus reducing the loculi. In carpel 4 this process has gone so far that the loculus has vanished, and the median region of the carpel is attached by the middle line of its ventral surface to the infolded and fused margins of its two half placentae; and in carpels 5 and 6 these marginal outgrowths have closed in a hood-like manner over the apex of the median region, so that it is no longer visible. As the loculi disappear, the marginal regions of the carpels thus take on an exaggerated development; they not only form the stigmatic crown itself, but also the outgrowths which produce the basal rim seen in transverse section in Fig. 2, c3a and c4a, and in longitudinal section in Fig. 3, A1 and A2.

Before leaving *P. Argemone* we may notice one point of minor importance. This is the occurrence of downwardly directed flanges from the sterile regions of the placentae high in the ovary. These can be seen cut transversely in Fig. 2, c2. The obliquity of the section reveals, on the left, two flanges continuous with the fertile placentae, and, on the right, four flanges cut in their free regions. The same structures can be seen in longitudinal section in Fig. 3, A1 and A2.

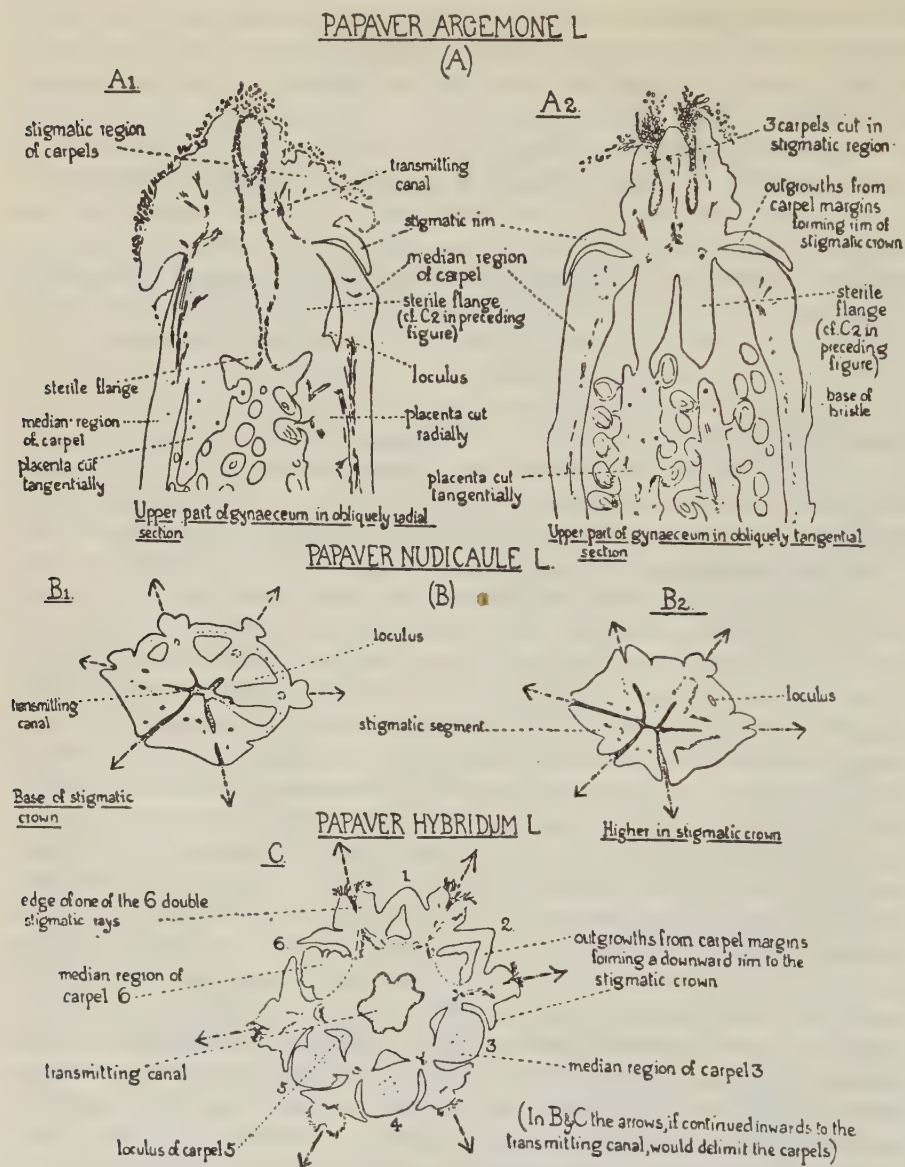


FIG. 3. A₁ and A₂, *Papaver Argemone* L. Sections from a slightly oblique longitudinal series through the upper part of a gynaeceum from an unopened bud, Camb. Bot. Gard., 25 June 1936 ($\times 14$). A₁, approximately radial; A₂, tangential. B₁ and B₂, *P. nudicaule* L., sections from a slightly oblique transverse series through a young gynaeceum at the stage at which differentiation of the ovule integuments has only just begun. Camb. Bot. Gard., 3 Aug. 1928 ($\times 14$). Here, and in c (below), the arrows, if continued back to the transmitting canal, would delimit the carpels. C, *P. hybridum* L., section at the base of the stigmatic crown from a transverse series through a gynaeceum, Camb. Bot. Gard., 28 July 1928 ($\times 14$).

Serial sections of the gynaecea of *Papaver nudicaule* L., *P. hybridum* L., and *P. Rhoeas* L. were cut for comparison with *P. Argemone*; they were found to agree with this species in all essential points. Fig. 3, c, passes through the base of the stigmatic crown in *P. hybridum*. Being slightly oblique, it shows the vascular median regions of carpels 3, 4, and 5, with the loculi on their inner faces, while in carpels 6, 1, and 2 the upper limits both of the loculus and of the vascular tissue of the median carpellary region have been passed. It will be seen that the ventral (inner) faces of the median regions of these three carpels are, at this level, united with the stigmatic crown. The arrangement of the elements makes it possible to distinguish the tissue of the median region of the carpel from the tissue of the marginal regions, and this boundary is indicated for these three carpels by dotted lines; but there is perfect tissue-continuity between the tips of the median regions of these carpels and the stigmatic crown. Fig. 3, c, also shows the development of the stigmatic rim, and the early stages in the division of the placentae and their bundles. In this diagram, and in those of *P. nudicaule* (B1 and B2), the arrows, if carried inwards to the transmitting canal, would mark the boundaries between adjacent carpels.

The sections of *P. Rhoeas* drawn in Fig. 4 may be used to illustrate certain special points which were not demonstrated for *P. Argemone*. One of these is the history of the vascular structure in the base of the gynaeceum and in the placentae. Fig. 4, A1, is cut at a level at which the bundles of the uppermost stamens have not yet left the axis. The central cylinder has ten residual bundles, which are perfectly distinct as regards their lignified xylem, but which form a ring by a continuity which chiefly concerns the phloem and the sheath tissue. In A2a we pass into the stalk of the gynaeceum, which corresponds, on a small scale, to the gynophore of the Capparidaceae. The ten residual bundles are now free from one another, and there is no vascular tissue between them. In A2b (the bundle x in A2a on a larger scale) we find that, outside the sheath cells, which are large, with highly stainable walls, there are groups of active elements, rich in contents, which, at a higher level, will give rise to vascular strands. Similar groups are seen in E1b and E2. In A3 the bundles for the gynaeceum are already marked out. Between the ten lignified bundles (which are the fused marginals of adjacent carpels, from which the placental supply will originate) there are extremely delicate non-lignified strands for the main regions of the carpels. These strands arose *de novo* in the parenchyma, and at this level they are not connected with the fused marginals. In A4 we come to the first appearance of the carpel loculi, which surround the vestigial non-vascular apex of the floral axis; sections showing this feature have been obtained from four other gynaecea. Fig. A5a is cut at 70μ above A4, but below the fertile region of the ovary. The groups of active cells, belonging to the surface tissue of the bundles, which were noticed in A1b, are now giving rise to unlignified strands which connect with the bundles of the median regions of the carpels—also at this level unlignified.

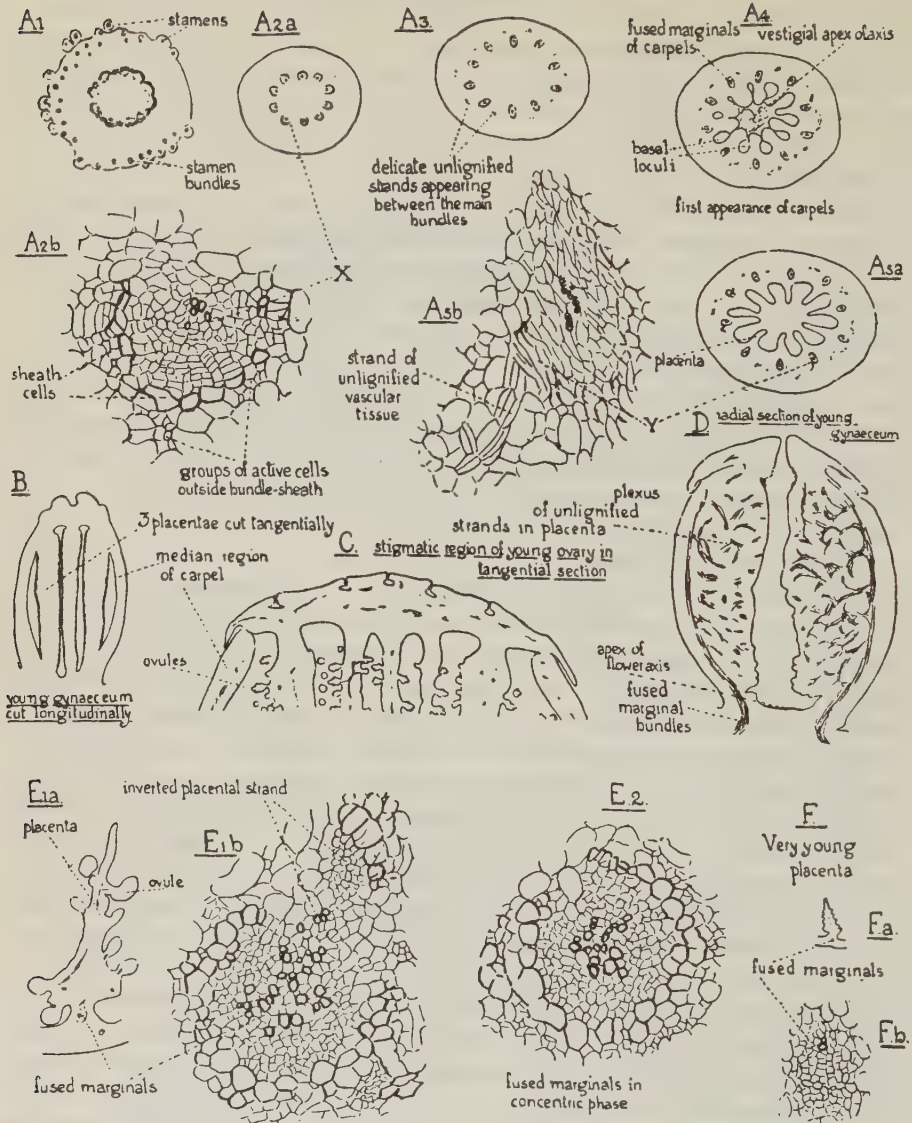
PAPAVER RHOEAS L

FIG. 4. A-D, *Papaver Rhoeas* L. A1-A5, sections from a transverse series through the lower part of the gynaeceum of an unopened bud, gravel pit, Camb. Univ. Farm, 24 May 1936 ($\times 14$, except A2b and A5b, which are $\times 193$ circa). A1, level at which there are still stamen bundles in the floral axis. A2b, bundle x in A2a on a larger scale. A4, actual base of gynaeceum. A5a, sterile bases of placentae. A5b, bundle y in A5a on a larger scale. B, longitudinal section from a series through a very young ovary, Whittlesford, 28 May 1936 ($\times 23$). Three placentae are cut tangentially, and the median regions of two carpels are cut more or less radially. The gynaeceum is so young that no ovules have yet been produced. The shaded edges of the placentae represent the border of cells rich in protoplasm from which the ovules will be

The fused marginals are slightly oblique. Fig. A5b shows one of these bundles, y, on a larger scale; it is giving off an unligified branch to the south-west.

Even in extremely young ovaries of *P. Rhoeas*, from the base the fused marginal strands tend to be slightly concentric (Fig. 4, fb). In older stages this feature is emphasized, the xylem being placed centrally with the phloem surrounding it, and the sheath closing more or less completely round the bundle (Fig. 4, E2). The result is that when a strand from such a bundle is given off towards the placenta, the phloem of the branch bundle is naturally turned towards the placenta and the xylem towards the parent bundle (Fig. 4, E1a and E1b).

The nature of the vascular system within the placenta cannot be understood from transverse sections, but its general scheme is shown in Fig. 4, D, which is a radial section through a gynaeceum so young that the rudimentary ovules have not yet produced integuments. It will be seen that a very elaborate plexus of strands arises from the fused marginal bundles and penetrates the tissue of the placenta. At the stage figured there is little lignification except in the main bundles.

The tissue-continuity between the median regions of the carpels and the stigmatic crown has already been demonstrated in transverse sections of *P. Argemone* and *P. hybridum*. In Fig. 4, B and C, longitudinal sections of a young gynaeceum of *P. Rhoeas* are drawn to show this continuity from a different aspect; it is only at a later stage that it is interrupted by the formation of the dehiscence pores.

The two genera most nearly allied to Papaver are *Argemone* and *Meconopsis*. Serial sections of the gynaeceum of *Argemone mexicana* L. are illustrated in Fig. 5, A1–A9, and the stigmatic crown is shown as a solid object in C1 and C2. The general structure is essentially similar to that of Papaver, so our description may be limited to the vascular supply, in which certain critical features can be seen more clearly than in Papaver. In the A series in Fig. 5 the bundle x and its products are marked in each diagram, so that its history can be followed into the stigma. As compared with Papaver, the placentae in the fertile region of the ovary, and the base of the apical sterile region, project little into the cavity (Fig. 5, A1), and the vascular supply for the placenta scarcely becomes detached from the fused marginals. A2a and A2b in Fig. 5 show two inverted bundles given off on the placenta side, which have remained

developed. c, tangential section from a series through the top of a gynaeceum, Whittlesford, 28 May 1936 ($\times 14$); the ovules are so young that they are still atropous, but rudiments of both integuments have appeared. D, approximately radial section from a longitudinal series through a young gynaeceum, Whittlesford, 28 May 1936 ($\times 14$); the ovules have not yet developed the rudiments of integuments. E1a, one placenta from a transverse series through the gynaeceum of an open flower, gravel pit, Camb. Univ. Farm, 24 May 1936 ($\times 14$). E1b, fused marginal strands from E1a ($\times 193$ circa). E2, fused marginals associated with another placenta in the same section as E1; this bundle is not, at the moment, giving off a strand to the placenta ($\times 193$ circa). Fa, one placenta from a transverse series through the gynaeceum of an unopened bud, gravel pit, Camb. Univ. Farm, 24 May 1936 ($\times 14$). At this very young stage the ovules are mere prominences. fb, fused marginals of Fa ($\times 193$ circa).

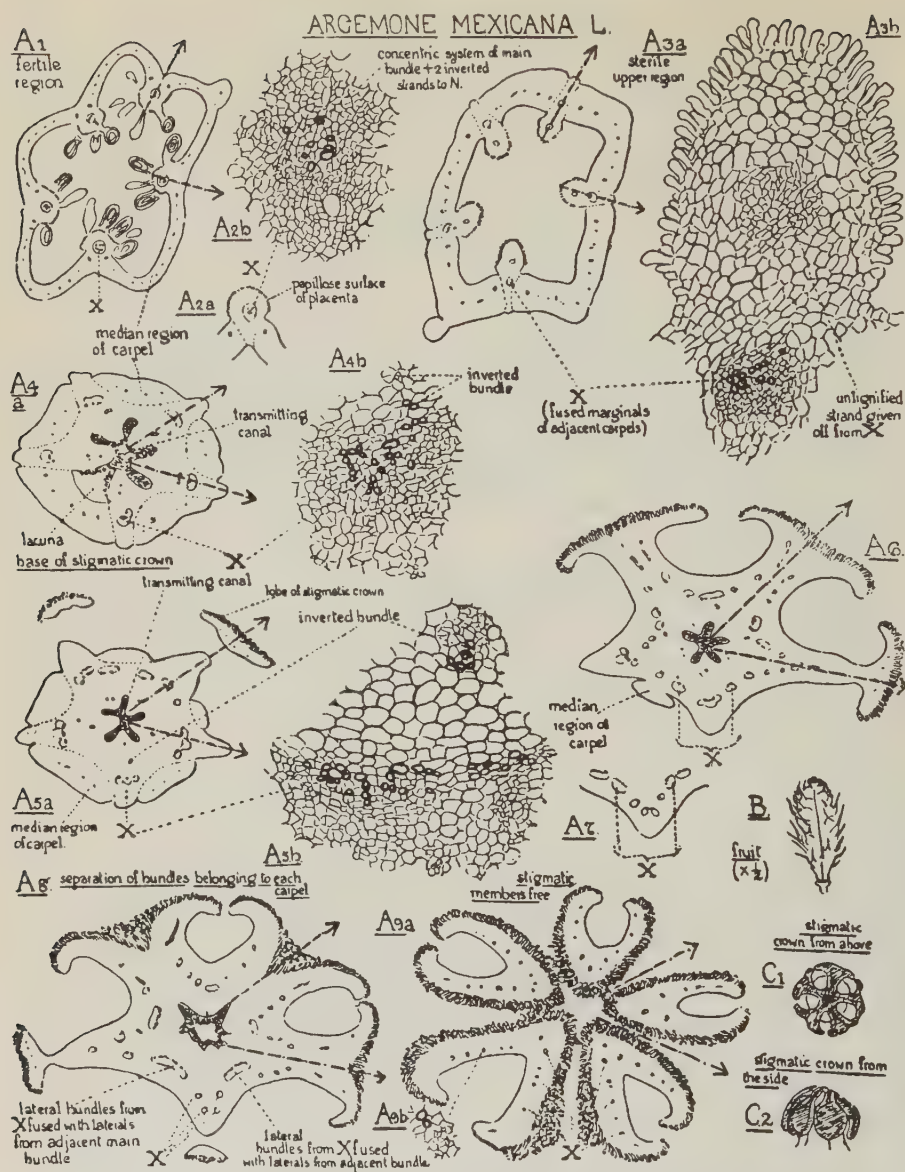


FIG. 5. *Argemone mexicana* L., Camb. Bot. Gard., 13 July 1936. A1–A9, sections from a transverse series through the upper part of a gynaecium; one carpel is delimited by arrows. A1, fertilized region of ovary ($\times 14$). A2a, a placenta cut at a slightly higher level where it is sterile ($\times 23$). A2b, fused marginal bundles in A2a ($\times 193$ circa). A3a, upper sterile region of ovary ($\times 23$). A3b, placenta and bundle x ($\times 193$ circa). A4a, extreme top of ovary where it passes into stigmatic cap; section slightly oblique, and one lacuna only seen ($\times 23$). A4b, x in A4a ($\times 193$ circa) to show an inverted bundle being given off. A5a passes through apices of median regions of carpels, above the tips of the vascular bundles of three of them ($\times 23$). A5b, x in A5a ($\times 193$ circa); the main bundle is spreading and dividing, and the inverted bundle

in contact with the fused marginals to form a concentric plexus. In the higher sterile region of the ovary, however, an unlignified strand passes into each placenta (Fig. 5, A3*a* and *b*), while, in the base of the stigmatic crown, corresponding bundles, which are lignified and show inverted orientation, move away to some distance from the fused marginals (Fig. 5, A5*a* and *b*). In A6–A9 the process in which *x* shows its compound character can be followed; its components become distributed between the adjacent carpels to which they belong. In the stigmatic members the xylem of each bundle is directed towards the papillose surface (A9*b*).

Meconopsis is slightly modified from the Papaver type by the fact that the stigmatic crown is raised on a style. A few sections from a series through the distal portion of a gynaeceum of *M. aculeata* Royle are drawn in Fig. 6. The upper sterile parts of the placentae are shown in Fig. 6, A1. The tip of the median region of each carpel has a slight 'shoulder' at its attachment to the style; two of these projections are cut in their free region in A2. A3 passes through the style, and it is seen that the central transmitting canal has four arms *in the median planes of the carpels*. A4 is higher in the style; at this level the sectional form of the transmitting canal has changed, and it now shows four arms *in the planes between the carpels*. These arms are the first indications of the disjunction of the four carpels. In A5 we reach the stigmatic region in which the separation of the four carpels is complete.

III. DISCUSSION

In contrast with the theory of the gynaeceum of Papaver generally used by systematists, which has been applied to the minute structure in the preceding pages, another view, sponsored by Kerner and Oliver (1895), has been revived in recent years by Saunders (1930), &c., and Dickson (1935). According to this view the parts which are called in the present paper the median regions of the carpels, are interpreted as an outer whorl of sterile carpels, while the placentae represent an inner whorl of fertile carpels. Some of the evidence adduced by Dickson, who has given the most detailed exposition of this theory, must now be considered. In certain critical points the observations in the present paper differ somewhat from those recorded by that author. She states that in Papaver the 'expanded carpels' [median regions of the carpels, in the terminology used here] '*are never fused with the stigmatic disc*, for the disc can always be dissected from the capsule without causing any injury to the tips of the expanded carpels. Specimens at every stage of growth were examined, and this was always so in *Papaver Rhoeas*, *P. Argemone*, *P. hybridum*, *P. somniferum*, *P. orientale*, *P. bracteatum*.' Such

has been detached. A6, base of stigmatic crown ($\times 23$). In A6 and A7 all the bundles within the bracket are derived from *x*. A7, further division of *x* ($\times 23$). A8 and A9*a* ($\times 23$). A9*b*, one of the bundles in A9*a* ($\times 193$ circa) to show that the xylem is turned towards the papillose surface. B, fruit ($\times \frac{1}{2}$). C1, gynaeceum as a solid object seen from above. C2, a similar stigmatic crown seen from the side. C1 and C2, both enlarged and prickles removed.

observations might be held to support the idea that the placenta, and the median regions of the carpels, belong to different members; it may be doubted, however, whether it is safe to base negative conclusions about tissue connexions upon evidence of this type. The sections figured in the present

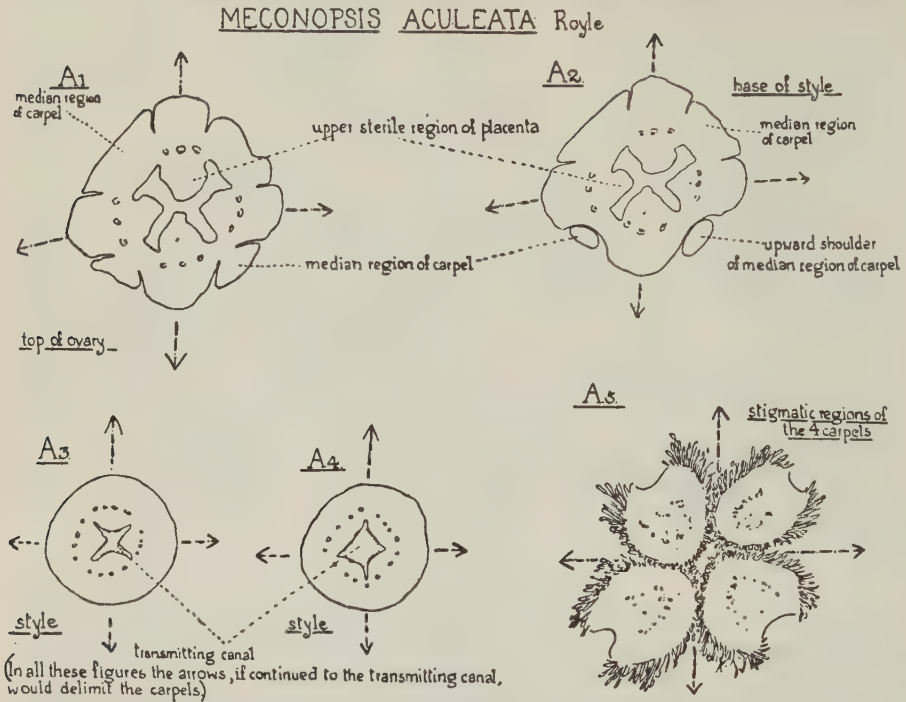


FIG. 6. *Meconopsis aculeata* Royle. Sections from a transverse series through the upper part of a gynaecium, Camb. Bot. Gard., 26 June 1936 ($\times 23$). The arrows in each diagram, if carried to the transmitting canal, would delimit the carpels.

paper show that in the young gynaecium of *P. Argemone* (Fig. 2, c4a, carpel 4; Fig. 3, A2), *P. hybridum* (Fig. 3, C, carpels 6, 1, 2), and *P. Rhoëas* (Fig. 4, B and C) there is *perfect tissue-continuity* between the median region of the carpel and the stigmatic crown. At these young stages the stigmatic crown could be removed only if the delicate connexions were torn away; in the process of dissection such tearing might easily happen without being perceptible. Again, in describing the stigmatic structure, Dickson states that 'the vascular bundles of the contracted carpels' [the fused carpel margins with their placental outgrowths, in the terminology used here] 'remain unchanged throughout their course. When they reach the top of the ovary-wall they bend inwards, almost right angles, and run along underneath the stigmatic rays.' This, as it stands, might also be accepted as evidence for the interpretation of the marginal and placental regions as distinct carpels, but a different picture is given by the observations described in the present

paper. In my preparations the fused marginal bundles do not 'remain unchanged throughout their course', but each invariably separates into two halves in the base of the stigmatic region, one half passing into the stigmatic member on either hand. This is illustrated for *Papaver Argemone* in Figs. 2, c3a and b, c4a and b; while the process can be followed more easily for *Argemone mexicana* in Fig. 5, in which the bundle x can be traced throughout the A series.

In recent theories of the constitution of the gynaecium in the Cruciferae and Papaveraceae, stress has been laid on the inverted character of the bundles supplying the placentae, and their orientation has been accounted for by supposing them to be the ventral strands of the 'contracted carpels' (e.g. Dickson, 1935). The inversion of these strands can, however, be explained quite simply on mechanical grounds. It must be remembered that each placenta is immediately internal to one of the strands which are here interpreted as fused marginals. Exactly how such a strand can supply bundles to the placenta, which is on its own radius, is a special case of the general problem of how a collateral bundle can give off a branch on its xylem side. In *Papaver Rhoeas*, as we have seen, this problem is solved by the bundle becoming concentric (Fig. 4, E2); when a portion of it is detached on the side towards the placenta, it thus naturally has its xylem turned towards the parent strand, and its phloem away—in other words, it is 'inverted' (Fig. 4, E1b). The same thing occurs in *Argemone mexicana*, but here the inverted strands tend to remain associated with the fused marginals to form a concentric plexus (Fig. 5, A2a, A2b), though in the base of the stigmatic cap they may become free (A4a, b; A5a, b).

The occurrence of 'inverted' bundles in the placentae is only one of the aberrant results that may follow when one collateral bundle has to supply a vascular system for a structure internal to itself on the same radius of the shoot. It has been shown in a previous paper (Arber, 1932; see also 1937a) that the two-bundled character of the stamen filaments in *Hypecoum*—which has sometimes been interpreted as indicating that these stamens represent an ancestral pair—is also due merely to a special type of bundle-branching associated with the superposition of a stamen upon a petal.

It will, I think, be recognized from the descriptions and figures in the preceding section of this paper that the gynaecium of *Papaver* can be described in a consistent fashion on the theory that it is formed from a single whorl of carpels, all of the same type, and bearing ovules on placental outgrowths from the united margins of adjacent members. On analysis, the peculiarities of the *Papaver* gynaecium are all found to arise out of the reduction of the median region of each carpel, and the corresponding overgrowth of its margins. The carpel shows that 'sorte de tendance à la trilobation' detected by Lignier (1911) in the gynaecium of *Glaucium*. The median regions of the carpels in *Papaver* do not extend to the apex of the gynaecium, but are completely overarched at the tip by a hood formed from the marginal regions. The

vascular system of the median region is reduced in correspondence with the external reduction, while the fused marginal bundles are highly developed in correlation with their function as the supply channels for the ovule-bearing placentae.

Within the Papaveraceae it would be hard to find a genus with gynaecium structure more remote from that of *Papaver* than *Platystemon*. We have

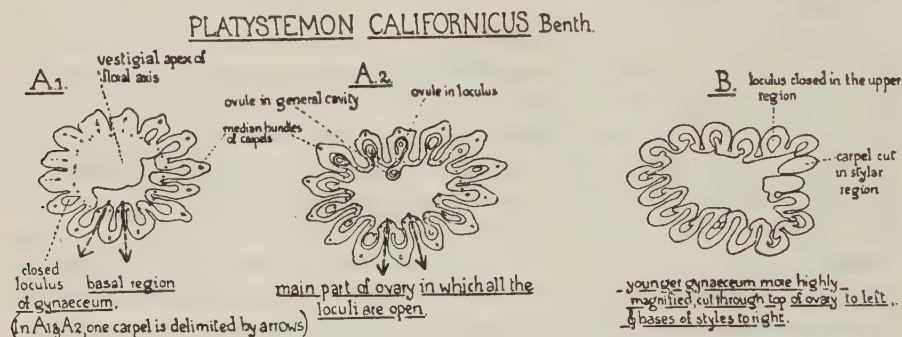


FIG. 7. *Platystemon californicus* Benth., Camb. Bot. Gard., 5 June 1936. A₁ and A₂, sections from a slightly oblique transverse series through a gynaecium ($\times 14$). B, section from a slightly oblique transverse series through a very young gynaecium, showing to the right the base of the stylar region ($\times 23$).

shown that the theory here adopted gives a consistent explanation for *Papaver*, when this genus is considered in itself, and now—as a further test of the efficiency of the theory—we will apply it to the comparison of *Papaver* and *Platystemon*. In the base of the ovary of *Papaver Rhoeas* (Fig. 4, A₄) the carpels, which at their first appearance have closed loculi (cf. also Fig. 1, B₁), surround the vestigial apex of the floral axis. Such sections may be compared closely with that of *Platystemon californicus*, cut at a corresponding level, which is drawn in Fig. 7, A₁. If the marginal bundles of adjacent carpels were united, and the ovules were borne on a placental outgrowth from the fused margins, we should arrive at a scheme of structure recalling *Papaver*. Fig. 7, A₂, with its open loculi, if it were so modified, might be compared with the section of *Papaver Argemone* drawn in Fig. 2, C₁. Fig. 7, B, is cut through the upper part of a very young ovary of *Platystemon*, and we see that, just before the carpels become stylar, the loculi repeat the closure which we noticed in the base. This closure of the loculi at the tip of the ovary can be paralleled in *Papaver*. The stigmatic members of *Platystemon*, like those of *Papaver*, are each formed from a single carpel only; but those of *Platystemon* differ from those of *Papaver* in incorporating the median as well as the lateral regions of the carpel. We thus see that, though at first glance the gynaecium of *Papaver* seems remote from that of *Platystemon*, the fundamental relation of these superficially dissimilar types becomes clear when they are considered in the light of the theory here adopted.

IV. SUMMARY

This study is based upon serial sections of the gynaecia of *Papaver Argenum* L., *P. Rhoeas* L., *P. hybridum* L., *P. nudicaule* L., *Argemone mexicana* L., *Meconopsis aculeata* Royle, *Platystemon californicus* Benth., and other Papaveraceae. It is shown that the minute structure offers no support to the hypothesis that the gynaecium of Papaver includes two types of carpel—sterile and fertile (see also Arber, 1937a). On the contrary the evidence favours the view that the gynaecium consists of a single whorl of carpels, which are identical in type, and are equal in number to the placentae, and to the duplex stigmatic rays. In the ovary adjacent carpels are fused marginally, and the placentae represent outgrowths from these united edges; the placentae are thus duplex in origin. The inverted bundles of the placentae owe their inversion to an anatomical necessity; in morphological argument no stress, therefore, can be laid on this peculiarity. The carpels separate in the stigmatic crown by the splitting of each placenta along its median plane. The fused marginal bundles divide, and their components are distributed to the carpels on either hand to which they belong. The free surfaces produced by the splitting of the placentae bear papillae; each stigmatic ray hence represents the adjacent faces of two carpels. The median regions of the carpels are much reduced and take no part in the formation of the stigmatic crown. It is not, however, until a relatively late stage that the apices of the median regions of the carpels break away from the base of the stigmatic crown, thus forming the dehiscence pores. Each stigmatic member stands directly above the median region of the carpel to which it belongs; it is formed exclusively from the marginal and placental regions of that carpel, which overarch the reduced median region.

The structure in the allied genera *Argemone* and *Meconopsis* differs in no essential respect from that in *Papaver*.

A comparison with *Platystemon*—chosen as the member of the Papaveraceae in which the gynaecium differs most obviously from that of *Papaver*—shows that the theory outlined above makes it possible to relate these two extreme types, and thus to bring the gynaecium structure of the Papaveraceae under a unified conception.

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The Effects on the Rates of Transpiration and Absorption of Small Variations in the Pressure in the Xylem Tracts¹

BY

E. C. HUMPHRIES, Ph.D.

With five Figures in the Text

INTRODUCTORY

ALTHOUGH it still appears to remain debatable whether tensions of any considerable magnitude are set up in the water columns of actively transpiring trees, it is at least generally agreed that the pressure in the tracts may become reduced below that of the atmosphere. A pressure deficit is then said to be created (Haines, 1928). The effects of relatively high pressure deficits upon transpiration and absorption rates are in course of investigation by Haines (1935, 1935*a*, 1936), but only a preliminary communication (Haines, 1936*a*) has so far appeared on the effects of very small deficits. Since these appeared anomalous it was suggested to the writer that they should be reinvestigated. The reinvestigation was purposely carried out without knowledge of the results already obtained by Haines (1936), but the same unexpected general conclusions were reached in most cases. In the experiments here described the effects of both small deficits and small positive pressures have been dealt with together, since it was found that the latter could be conveniently investigated with the same apparatus.

EXPERIMENTAL

Series I.

Preliminary experiments were carried out with a simple form of apparatus. This consisted of a long glass capillary tube, approximately 1 metre in length and of 1 millimetre bore. At the top of this tube was fitted a cylindrical funnel, while the bottom was connected by a length of pressure tubing with a glass cup containing mercury. A rubber stopper with two holes was fitted into the top of the funnel. Through one hole the stem of a tap funnel passed, and through the other the experimental branch.

A satisfactory joint between the rubber stopper and the stem for the range of pressure used was made in the manner described by Haines (1936*a*). A scale graduated in millimetres was placed behind the vertical glass capillary

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tube. A quantity of mercury was introduced into the glass cup, pressure tubing, and capillary tube. The rest of the apparatus was filled with water.

In an actual experiment, a positive pressure of approximately 40 cm. of mercury was applied to the cut end of the branch by raising the mercury cup and noting the difference in level between the mercury menisci, the tap being closed. The rate of absorption in successive minutes was observed by reading the height of the mercury meniscus in the capillary tube. From these readings the pressure at any given minute could be calculated, allowance being made for the column of water above the mercury. During an experiment the branch was subjected to constant illumination supplied by 100-watt lamps.

By absorption of water the plant itself reduced the pressure at its cut end, so that during the course of each experiment it experienced a change from a positive pressure to a pressure deficit. It was not found possible in most cases to obtain reliable results below -30 cm. of mercury pressure as air was drawn through the plant.

The kind of result obtained is shown by the figures for a branch of *Acer pseudoplatanus* plotted in Fig. 1. The rate of absorption is given in terms of meniscus movement in centimetres per minute. Absorption rates are plotted against time as the tract pressure gradually falls. It can be seen from the graph that after the plants have been absorbing water for some considerable time and the tracts are actually experiencing a pressure deficit the absorption rate increases for a period, although at the same time the pressure deficit is also increasing. In other experiments with *Acer* definite increases in the absorption rate were found when the pressure in the tracts was well below that of the atmosphere.

Similar experiments were carried out with branches of *Tilia Europea*, and here also there was a slight increase in the absorption rate of the cut end of the branch when the pressure in the tracts had fallen below that of the atmosphere. The results of four such experiments are shown in Table I.

In a third set of experiments branches of *Ligustrum* sp. were used. In all experiments with this material there was a continuous fall in the absorption rate as the pressure in the tracts was reduced. There was no approach even to a period when the absorption rate remained constant. Results from typical experiments are given in Table II.

Thus contrary to expectation, the absorption rates in the case of *Acer* and *Tilia* are shown to increase after subjection to small pressure deficits, while with *Ligustrum* no such anomalous effect is observed.

Series II.

Although the varying deficits set up by the above method of experimentation are no doubt more comparable with those set up in nature, it appeared nevertheless desirable to investigate also the effects of constant deficits. The absorption rates under constant deficits were determined by means of the

special form of potometer shown in Fig. 2. The potometer reservoir, A, carrying the plant and tap funnel for refilling in its upper end, communicated below through a three-way tap, B, with the similar glass bulbs, C and D, fixed on a wooden mount, E. Of these, C was connected by a long piece of pressure

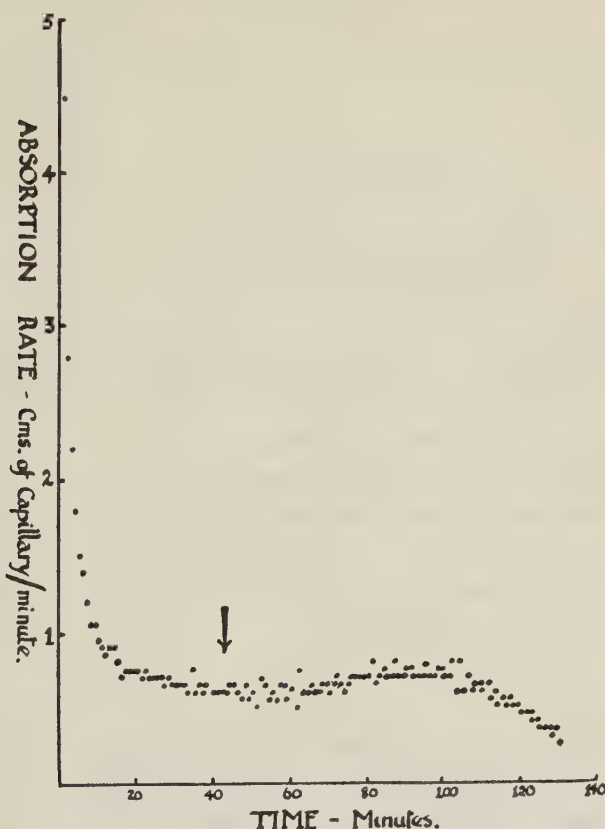


FIG. 1. *Acer pseudoplatanus*. Plotted results of an experiment to show changes in absorption rate with time during decreasing tract pressure. The pressure in the tracts is at first positive, but changes to a gradually increasing pressure deficit as the plant absorbs water. The point at which the pressure becomes atmospheric is indicated by the arrow. Note the rise in the values as a deficit is established.

tubing with the mercury reservoir, F, which could be raised or lowered to control the pressure in A, and D was connected indirectly with the capillary tube, G, in which the absorption rate was measured by the travel of a mercury meniscus over the scale, Q, in the usual way. On account of the varying pressure required in A, and set up by raising or lowering the mercury reservoir, F, provision had to be made for the scale tube, G, to be moved correspondingly up and down. Preliminary experiments showed that if it were merely connected by a length of pressure tubing in the same way as F, serious errors and difficulties arose through the extensibility of the tubing. These were

TABLE I

Amounts of Water absorbed by Branches of Tilia Europea during Successive Five-minute Intervals in Scale Divisions. (In the actual experiments the rate was observed at intervals of one minute)

The pressure was gradually reduced by the plant itself during absorption (see p. 62). Note the rise following the vertical stroke in each experiment.

Experiment 1.

10.65	8.1	6.1	4.55	3.4	3.1		2.95	3.05	3.10	3.00	2.85	2.80	2.85	2.65
				2.70	2.65		2.50	2.50	2.35	2.10				

Experiment 2.

34.0	5.9	3.2	2.95	2.85	2.1	2.0		2.0	2.05	2.15	2.20	2.10	2.10	2.05
								2.00	2.00					

Experiment 3.

34.25	8.55	4.1	4.05	3.9		3.65	3.8	3.7	3.85	3.75	3.60	3.40	3.20
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Experiment 4.

26.8	6.2	3.2	2.85	2.65	2.40	2.40	2.35		2.25	2.30	2.20	2.20	2.15
		2.10	2.15	2.10	2.20	2.10	2.20		2.00	2.00	1.65		

TABLE II

Amounts of Water absorbed by Branches of Ligustrum sp. during Successive Five-minute Intervals in Scale Divisions. (In the actual experiments the rate was observed at intervals of one minute)

The pressure was gradually reduced by the plant itself during absorption (see text, p. 666). In expt. 7 the corresponding falling pressures are also given.

Note the continuous fall in the values.

Experiment 5. 15.7 6.1 5.3 4.85 4.45 4.30 4.10 3.85 3.75 3.60 3.20

Experiment 6. 8.4 4.7 4.0 3.55 3.35 3.30 3.10 3.0 3.0 2.9 2.9 2.7

Experiment 7. Absn. rate: 9.25 5.6 5.15 4.7 4.4 4.25 4.05
Press. (cm./Hg): 26.7 21.3 16.5 12.2 — 4.2 —

Absn. rate: 3.95 3.95 3.50 3.35 2.90 2.15 1.75 1.25
Press. (cm./Hg): —3.3 — —7.5 — —15.9 — —19.7 —20.3

obviated by the arrangement shown in Fig. 2 in which the connexion with G is made by a thick-walled glass tube, H, approximately 1 metre in length, and two very short pieces of rubber pressure tubing, JJ. The tube, G, was mounted on a board, K, supported on a wooden arm, L, from the board, E. The wooden arm, L, was arranged parallel to the long capillary tube, H, and pivoted at points M, N, the same distance apart as the centres of the rubber connexions JJ. The fixings at M and N were by means of bolts and fly-nuts which conveniently allowed the board, K, and measuring tube, G, to be fixed at any required height relative to A. To reduce the strain on the arm, L, and to facilitate raising and lowering, the board, K, carrying the scale tube was connected by a cord passing over a pulley, O, with a counterbalancing weight, P. To carry out an experiment, the plant was inserted through the rubber stopper by the method referred to above (p. 665) and left for at least half an

hour to attain saturation. Readings of the absorption rate were then taken at atmospheric pressure. The tap, B, was set to place A and D in communication, and the height of K was adjusted until the mercury meniscus in G remained on the zero of the scale, Q, when the tap funnel was open to the air and the

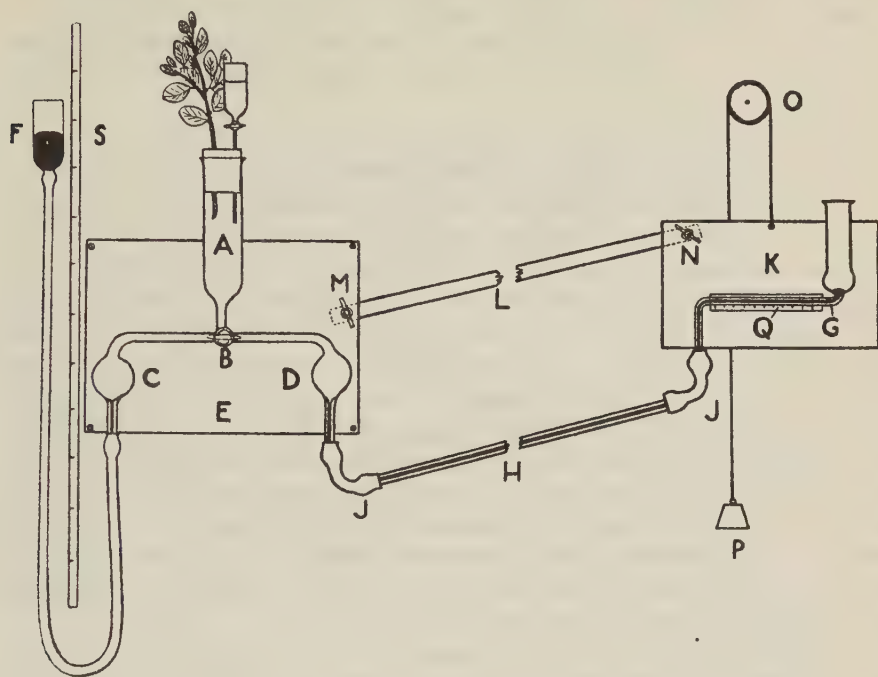


FIG. 2. Apparatus for following up absorption rates under constant positive pressures or constant pressure deficits. For explanation see text, p. 667 et seq.

contents of A therefore at atmospheric pressure. When the tap of the tap funnel was closed the mercury meniscus moved along the scale. The distance that the meniscus moved in one minute was noted. Ignoring the small pressure due to the water column in the tap funnel, as long as the mercury remained in the horizontal part of the measuring tube the pressure at the cut end of the branch was the same as that of the atmosphere. Successive readings could be taken by opening the tap of the funnel and allowing the mercury meniscus to return to zero on the scale in the usual way. When the rate of absorption at atmospheric pressure reached a steady value the pressure in A was raised by a small amount (e.g. 10 cm. of mercury) by raising the level of K, putting A, C, and D all into communication by means of the three-way tap, B, and raising the mercury reservoir, F, until the mercury meniscus again just reached the zero on the scale tube, Q. The exact pressure applied could then be read off by the difference between the levels of the mercury in F and C on the scale, S. To take a reading, the tap, B, was then set to allow communication only between A and D, and the rate of movement of the

mercury in the graduated tube, G, again noted. The pressure on the cut end could be maintained by raising the mercury cup from time to time and putting all limbs into communication, A being refilled between readings from C instead of from the funnel.

Readings of the absorption rate at constant pressure were taken at intervals, and when the rate became steady the pressure was again returned to atmospheric by lowering the mercury cup and scale-tube mounting and opening the top tap.

In order to obviate the risk of leakage past the three-way tap, B, between C and A or C and D, the pressure in C was maintained the same as that in A and D during experiments whether otherwise necessary or not. No leakage through B to the outside tended to occur. Throughout the experiments of this series the branches used were of *Tilia Europea*. Very consistent results were obtained, and it was therefore not considered necessary to perform a large number of experiments at each pressure. The pressures used were +40, +30, +20, +10, -10, and -20 cm. of mercury. The results are given in Table III and Fig. 3. Two experiments at each pressure are quoted.

On application of a positive pressure of 40 cm. of mercury the absorption rate increased to two or three times the original rate at atmospheric pressure, but rapidly fell again and reached a nearly constant rate (still above the normal) after approximately 90 minutes. On returning to atmospheric pressure the rate of absorption fell below the original at atmospheric pressure and then showed a tendency to increase. After this it remained at a constant value (Table III, expts. 8 and 9, and Fig. 3, *a*).

A parallel march of changes was obtained with positive pressures of 30 cm. mercury, except that the initial rise in the absorption rate on applying the pressure was not so great as at a pressure of 40 cm. of mercury (Table III, expts. 10 and 11, and Fig. 3, *b*).

When a positive pressure of only 20 cm. of mercury was used the rise in absorption rate was not so marked as in either of the preceding cases, and the subsequent falling off was also less rapid (see Table III, expts. 12 and 13, and Fig. 3, *c*). An excess pressure of 10 cm. of mercury caused only a slight rise in absorption rate and equilibrium was soon attained (Table III, expts. 14 and 15, and Fig. 3, *d*). Thus, as was to be expected, the initial absorption rate was greater the greater the pressure applied.

The experiments using pressure deficits of 10 and 20 cm. of mercury proved of interest because they substantiated the results of Series I. Reference to Table III, expts. 16 and 17, and Fig. 3, *e*, will show that on first applying a pressure deficit of 10 cm. of mercury the absorption rate fell slightly, but soon began to increase again and eventually exceeded the original rate at atmospheric pressure. On returning to atmospheric pressure the absorption rate further slightly increased. Although a pressure deficit of 20 cm. did not cause the absorption to exceed the original rate, after the deficit had been in operation the absorption rate slowly increased to a level somewhat above

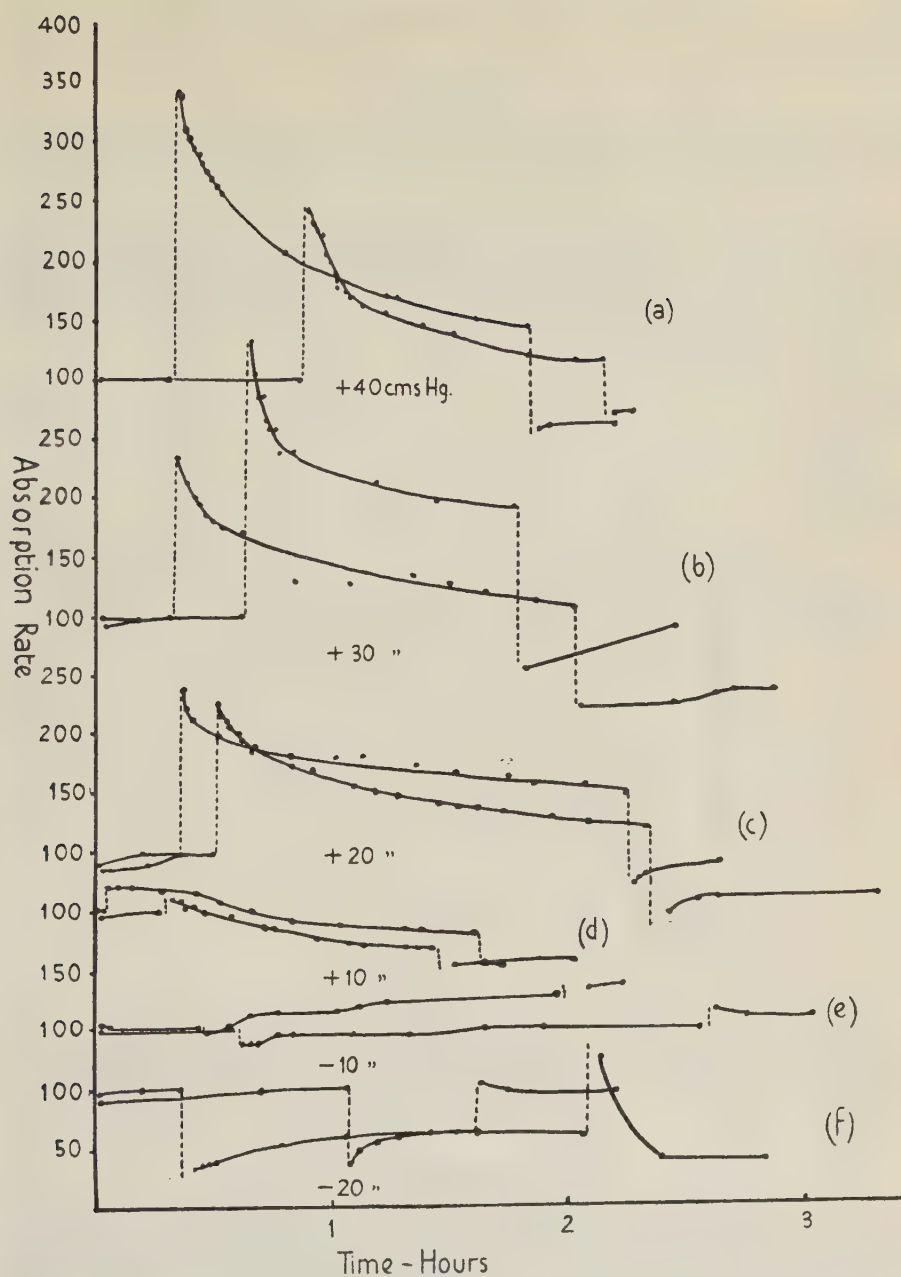


FIG. 3. Graphs showing changes in absorption rate with time under various constant positive pressures and constant pressure deficits.

that when the pressure deficit was first applied (see Table III, expts. 18 and 19, and Fig. 3, f).

TABLE III

Successive Readings of Absorption Rate in Scale Divisions on the Application and Release of Various Pressures artificially applied (see text, p. 670)

To economize in space the time relations have been omitted from the table but may be appreciated from Fig. 3, in which these results are plotted as graphs. All values are given, moreover, only as percentages of the original constant rates before artificial pressures were applied. In each experiment the first group of figures gives the initial rate, the second the successive rates observed (not necessarily at equal intervals of time) under the applied pressure, and the third the rates read after the pressure was released. Two experiments are quoted for each pressure.

Experiment	8	9	10	11	12	13	14	15	16	17	18	19
Pressure applied in cm./Hg	+40	+40	+30	+30	+20	+20	+10	+10	-10	-10	-20	-20
Initial absn. rates	100	102	98	99	92	100		96	100	99	100	100
	100	100	100	100	100	100	100	100	100	100	100	100
Absorption rates under pressures	341	242	235	333	229	227	122	113	97	88	34	38
	338	232	213	304	222	216	122	110	102	88	35	50
	310	227	200	286	212	211	122	105	111	88	37	55
	304	221	195	286	200	207	122	105	114	96	37	59
	296	205	184	265	190	200	118	100	116	96	56	62
	290	200	180	259	182	196	115	98	121	96	59	62
	282	189	176	259	182	189	109	88	125	97	62	62
	276	179	170	237	184	189	100	88	132	104	62	
	270	189	129	240	168	176	93	79		104		
	262	176	127	215	176	172	90	74		104		
	256	170	136	197	168	158	87	73				
	209	164	133	194	149	153	86	72				
	186	158	127		157	149	83	72				
	170	147	120		160	144		71				
	150	139	111		160	140						
	147	120	104		150	136						
		116				131						
		116				127						
						122						
Absorption rates after release of pressures	59	71	23	84	76	51	58	57	138	120	106	130
	62	74	27	91	81	64	61	59	142	117	100	93
	63		36		84	67		59		117	100	93
			38		95	67		59				
			38									

Series III

Since the foregoing experiments showed that a pressure deficit was normally followed by an increase in the rate of absorption, it was decided to determine also whether there was a corresponding effect on the rate of transpiration.

In order to investigate this, the transpiration rates were found by weighing, the plant being placed in a special kind of vessel (A, Fig. 4) suspended from a balance and flexibly connected through a reservoir, B, with the manometer, C, and a pump, by which the pressure of the water supply in A could be

controlled at will. The cut end of the branch was fitted through the single hole in the rubber stopper of the shorter limb of the J-tube, A (Fig. 4) by the method already referred to (p. 665). The longer limb of the J-tube was bent at right angles and drawn out into a fine process with a small bulb near the end to fit a length of bicycle-valve tubing by which it was connected to the

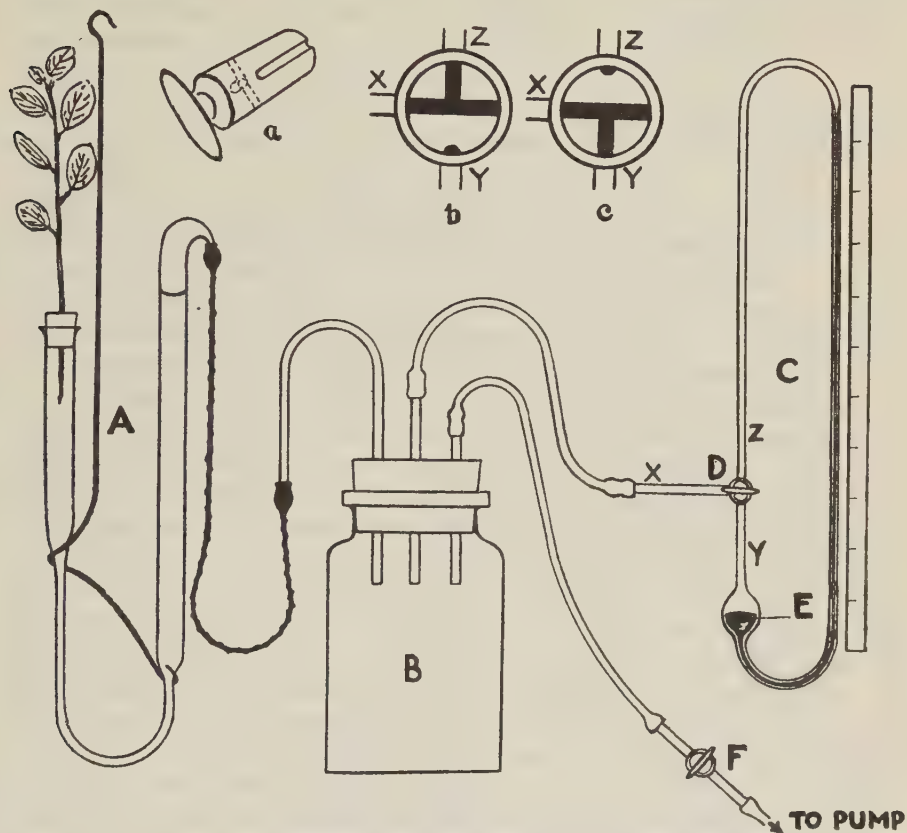


FIG. 4. Apparatus for following up changes in transpiration rate by the weighing method when the plant is subjected to constant positive pressures or constant pressure deficits. Insets: *a* shows the longitudinal groove ground in the key of the tap, *D*, of the manometer; *b* shows the setting of the tap for measuring reduced pressures, and *c* the setting of the tap for measuring increased pressures. For full explanation see text, p. 672 et seq.

glass reservoir, B, of approximately 200 c.c. capacity. A close spiral of thin Eureka wire was threaded into this valve tubing throughout its whole length in order to prevent its collapse under reduced pressure. The manometer was specially constructed to allow both positive pressures and pressure deficits to be read on the same scale. It consisted of a continuous loop of glass tubing (Fig. 4) with the bulb, E, and a three-way tap, D, in one vertical limb, and the other vertical limb constituting the scale tube. A longitudinal groove was ground in the back of the key of the tap as shown in Fig. 4, inset *a*, to allow

communication between the corresponding tube and the atmosphere. Thus for measuring positive pressures, x and y could be set into communication, z being open to the air via the groove; and to measure deficits x and z were set into communication, y being open to the air. The settings of the tap are shown in Fig. 4, insets b , c .

The whole apparatus was enclosed in a specially constructed transpiration chamber. Communication between the tap, F , and the pump was made by a piece of pressure tubing passing through a hole in the wall of the chamber, the pump being placed outside.

The transpiration chamber consisted of a wooden cabinet approximately 1.5 m. in height, 1 m. in length, and 0.6 m. deep fitted with sash windows at the front and sides so that the air could be freely admitted when desired. At the same time the plant inside the chamber was completely shielded from draughts. The cabinet was sufficiently large to allow experiments to proceed for several hours without any appreciable change in the relative humidity of the atmosphere inside. A balance capable of carrying 1 kilogram and sensitive to 1 centigram was mounted on the top of the transpiration chamber.

A suspension from the left arm of the balance carrying the vessel, A , hung centrally inside the chamber through a small hole in the roof, while a suspension from the right-hand beam of the balance carried a pan for weights and hung outside the chamber on the right-hand side. All weighing operations were therefore conducted without opening the chamber, the raising and lowering of the beam being effected by a system of levers operated from bench level.

An experiment was carried out in the following manner. A plant was fitted through the stopper of A (Fig. 4) in the usual way (cf. p. 665) and this fitted into the J-tube, which was already filled with water. The valve tubing was then connected to the other limb of the J-tube which was suspended from the balance arm, and then the rate of transpiration followed up by noting the times at which successive equal amounts of water (usually 0.1 gm.) were lost. When the rate of transpiration became constant it was assumed to have reached equilibrium under the new conditions and the water supply was then subjected to a certain pressure by means of the pump. This particular pressure could be maintained sensibly constant by occasional adjustment with the pump, the tap which communicated with the pump being otherwise closed.

Several experiments were performed in which the cut end of the branch was subjected to a constant positive pressure. The transpiration, however, appeared to be affected very little by the application of pressures up to 40 cm. of mercury. The apparatus was not suitable for pressures above this value.

As was foreshadowed in the first two series of experiments, by far the most interesting results were obtained when the plant was subjected to a small constant pressure deficit.

The most marked changes in the transpiration rate, like those in the absorption rate (Series I, p. 666), occurred with branches of *Acer*. In the

majority of experiments, as soon as the deficit was applied there was an immediate rise in the transpiration rate which sometimes continued for as long as an hour. The transpiration rate then fell steadily for approximately another hour, finally reaching a value of from 40 to 50 per cent. below the original rate at atmospheric pressure. When the pressure was restored to normal the transpiration rate began to rise again in most cases. Sometimes there was a secondary rise in the transpiration rate when the pressure was again raised to that of the atmosphere.

The figures obtained in three experiments on detached branches of *Acer pseudoplatanus* are given in Table IV and are represented graphically in Fig.

TABLE IV

Showing the Effects of Constant Deficits on the Rate of Transpiration of Cut Branches of Acer pseudoplatanus

Experiment No. Pressure deficit	20		21		22	
	20 cm./Hg.		30 cm./Hg.		30 cm./Hg.	
	Time in minutes.	Absn. rate.	Time in minutes.	Absn. rate.	Time in minutes.	Absn. rate.
	0	100	0	100	0	100
Deficit applied	10	108	11	112	10	100
	22	118	26	127	19	116
	41	109	43	138	27	128
	67	55	58	138	46	124
	72	47	75	122	58	135
			89	98	74	130
			103	74	84	94
			117	74	101	82
			137	69	125	73
					143	69
Deficit released	85	31	150	76	156	67
	107	41	164	76	180	71
	140	50	180	86	193	78
	164	75	196	104	218	78
	185	91	218	128	267	86
	197	80	234	150		
	214	68	245	164		
			255	127		
			265	99		
			272	99		

5, *a*. Out of twelve experiments with *Acer* by this method, only three failed to show a rise in the transpiration rate after the pressure deficit had been applied. It was not found possible to determine the cause of these discrepancies.

Similar experiments were carried out using branches of *Tilia Europea*. In the majority of cases a similar effect was observed, the rise in transpiration rate being obvious immediately the deficit was applied, but the subsequent fall in the rate was extremely rapid, and about an hour after the deficit was first applied, the transpiration rate was only about 30 per cent. of its original rate at atmospheric pressure. This is clearly shown in Fig. 5, *b*, which has been constructed from the readings in Table V.

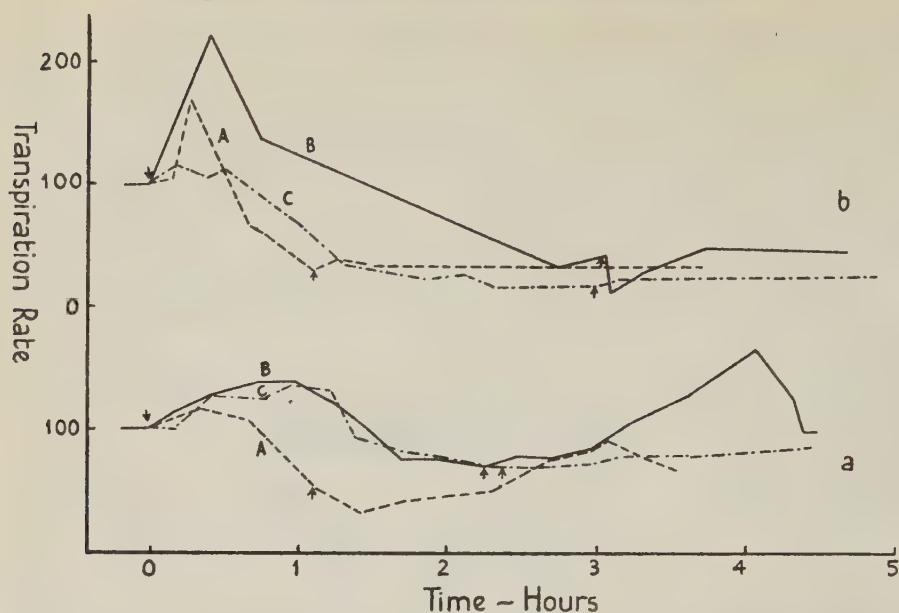


FIG. 5. a. *Acer pseudoplatanus*. Graphs plotted from the figures in Table IV to show changes in transpiration rate under various constant pressure deficits. A (expt. 20) at -20 cm. mercury, B and C (expts. 21 and 22) at -30 cm. mercury. The points of application and release of the deficit are indicated by the arrows. b. *Tilia Europea*. Graphs plotted from the figures in Table V to show changes in transpiration rate with time under constant pressure deficits. A = expt. 23, B = expt. 24, and C = expt. 25. Pressure deficit = 50 cm. mercury throughout.

TABLE V

Showing the Effects of Constant Deficits on the Rate of Transpiration of Cut Branches of *Tilia Europea*

Experiment No. Pressure deficit	23		24		25	
	50 cm./Hg.		50 cm./Hg.		50 cm./Hg.	
	Time in minutes.	Absn. rate.	Time in minutes.	Absn. rate.	Time in minutes.	Absn. rate.
Deficit applied	0	100	0	100	0	100
	9	104	29	220	11	127
	17	168	44	138	24	104
	28	114	143	52	30	112
	41	67	166	32	62	68
	47	59	184	41	78	38
	67	32			97	29
Deficit released					138	18
	75	38	186	12	190	21
	89	34	199	28	223	21
	138	31	225	48	292	23
	185	32	282	43	337	22

Out of fifteen experiments with cut branches of *Tilia*, five showed no increase in transpiration rate under a deficit. A few experiments were carried out on branches that were cut and kept in water a day before using so that

there would be no doubt that they were fully saturated at the time of experiment. Some failed to give any increase in transpiration rate during the experiment, but in the majority there was a small increase. This was not so marked as in the experiments on freshly cut branches which were only given an hour or so to attain saturation. It does not appear, however, that the effect is restricted to unsaturated branches or in any way an outcome of this condition.

DISCUSSION

The foregoing experiments have clearly established the fact that in the majority of cases the application of a pressure deficit to the cut end of a detached branch causes an increase in the transpiration and absorption rates of that branch. The effects found by Bakke (1915, 1918) in experiments with plants allowed to wilt in the laboratory will naturally be recalled to mind. In these cases, however, an increase in transpiration was not evident until about five days after wilting had begun. Nevertheless, the conditions in the tracts of the wilting plants must have been similar in kind, if not in degree, to those in the present experiments. Bakke accounted for the increase in transpiring power on the supposition that at a certain point the tension in the water columns became so great that they were suddenly ruptured, and this had the effect of decreasing the saturation deficit of the leaf cells and allowing temporarily increased evaporation to result. It seems that this assumption is open to question since an analogous increase in transpiration rate has been found in the present experiments, where a *known* pressure deficit was applied to the water columns, the deficits being so slight that they could not possibly cause the columns to break. Bakke's explanation cannot be applied in the present case moreover, since in the majority of cases recovery in the transpiration rate occurred when the pressure of the water supply was returned to normal, and this could not happen if the plant had permanently wilted.

Results more closely resembling those of the present series were obtained by Knight (1922). He found that on depriving a detached branch of its water supply, by closing a tap in a potometer, in the course of a few minutes the transpiring power of the leaves began to increase to a maximum which was attained in about forty minutes. The increase was about 50 per cent. above the rate of transpiration immediately before the tap was closed. This agrees very closely with the results obtained in Series III of the present investigation (pp. 675-6). It is evident that as soon as the water supply of the branch is stopped, as in Knight's experiments, a pressure deficit is inevitably set up in the tracts, because evaporation from the leaf cells continues in the absence of water supply. Strictly speaking, therefore, the conditions in the plant, both in the experiments of Knight and of Bakke, are similar, although the latter held that the increased transpiration only took place as a result of 'permanent wilting'. Knight attributed the increased transpiration rate to increased stomatal opening, but was of the opinion that 'undoubtedly there are other factors concerned in determining the extent of the increase'. He

found that in some cases the transpiration rate tended to increase before any stomatal movement became evident. Bakke also found no evidence for stomatal movement during the phase of increased transpiration.

In the experiments of the present series stomatal movement was not followed up by a porometer, but frequent tests by the injection method (Maximov, 1929) failed to indicate any change. However, since that method is open to serious objection in connexion with the present problem, a series of experiments are contemplated using a single leaf in which absorption, transpiration, and stomatal aperture will be followed simultaneously. Preliminary experiments have already shown that single leaves show the same sort of effect as that here recorded.

It is interesting to note that the first series of experiments (p. 666) show clearly that the rate of absorption is also affected by the deficit (though not to such a large extent as the transpiration) and tends to increase for some time after the deficit has been applied. This suggests that the immediate effect of applying the deficit is connected with the leaves and that increased absorbing power is a secondary outcome of this effect.

The results shown in Fig. 5 clearly indicate that the rise in transpiration rate is not necessarily proportional to the deficit applied since a rise of about 40 per cent. occurs when a deficit of either 30 cm. or 50 cm. is applied. It may well be that there is a critical deficit, below which no rise in transpiration will take place and above which the transpiration rate will increase but not necessarily proportionally to the pressure. In this connexion it is to be noticed that no increase in absorption rate tends to occur with detached branches of Privet when subjected to an increasing deficit. Consequently, the fact that the increase in transpiring power observed by Bakke did not occur until five days after the water supply was cut off from the roots does not necessarily imply that this effect is not similar to the present ones and those observed by Knight. Bakke used intact plants of *Helianthus annuus*, while Knight experimented on cut branches of *Eupatorium adenophorum* and *Peristrophe speciosa*. In the present series also, cut branches were used. The conditions prevailing in a cut branch are necessarily different from those in an intact plant. It seems likely that under the conditions used by Bakke, the supply of water to the leaves would be quite considerable for some time after the supply had been cut off from the roots. On the other hand, the water supply in cut branches would necessarily be restricted. Thus it would probably take longer for a deficit to be reached in the intact plant than in the cut branch. Moreover, the effect of increased transpiration might not be noticeable until a certain critical deficit had been reached. If this is the case, the present results and those of Bakke and Knight can be brought into line. It would evidently be premature to make any further suggestions on the mechanism of the effect before more data have been obtained.

SUMMARY

The effects of small pressure deficits on the rates of transpiration and absorption by cut leafy branches have been reinvestigated by various special forms of apparatus. The experiments also show the effects of small positive pressures in the conducting tracts.

Small positive pressures lead to an immediate and considerable increase in absorption, followed by a falling off towards a more or less constant value above the initial rate and depending on the pressure used. The fall to this value is rapid at first and more gradual later.

Small pressure deficits cause an initial rise in both absorption and transpiration rates in most cases, the effect on transpiration being the earlier and the more pronounced. The rise in absorption rate would therefore appear to be a secondary effect resulting from a more immediate effect upon transpiration consequent upon some altered condition in the leaves.

The anomalous effects of small deficits noted earlier by Haines are therefore confirmed, but are not quite the same in different plants (*Acer* and *Tilia*) and in some are not shown at all (*Ligustrum*).

Attempts are made to bring the results into line with those of Bakke and of Knight.

In conclusion my thanks are due to Professor Fritsch for providing facilities for carrying out the work and to Dr. Haines for helpful advice and criticism.

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The Occlusion of Pruning Wounds in Norway Spruce (*Picea excelsa*)

BY

ALEXANDER PATERSON, B.Sc.

With Plates XXV to XXVII and five Figures in the Text

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I. INTRODUCTION

IT is evident from the literature that, when a forest tree is pruned, the time taken for complete occlusion of the wound depends on several factors. Amongst these the most important appear to be the rate of radial growth in the stem, the length of the snag, and the diameter of the branch. There seems to be some doubt as to the merits of live-pruning as opposed to dead-pruning. It is claimed by some authorities that the pruning of live branches leads to the entrance of fungi, the formation of resin pockets, and to a reduction in the vigour of the tree. But Curtis (1936) points out that when a dead branch is pruned an opportunity is afforded for fungi to enter the tree through the entire area of the dead branch stub; also resin pockets are formed in the case of dead branch cuts due to the presence of the pitch mass borer (*Parharmonia pini* Kill.) and the pitch midge (*Retinodiplosis* sp.). In this country the problems associated with pruning are only of practical importance in connexion with softwoods, and this study has therefore been limited to this type of wood. It was also thought advisable to limit this investigation further to a thorough study of one species (Norway spruce), in which occlusion is exceptionally slow. It is hoped that the results obtained from this study may be applied to other softwoods later. Studies are being made elsewhere by Donald (1936) on the importance of various features such as branch diameter, length of snag, &c., and in consequence no attempt has been made in the following

investigation to study these features. Attention has been directed to the manner in which occlusion takes place. During the process of the research it soon became evident that there are marked differences in the manner in which occlusion takes place in live-and dead-pruned branches and that these differences are likely to affect the grain of the wood that is formed around the knot. The process of occlusion has been studied both macroscopically and microscopically. The arrangement of the tissues at the time of pruning, and their subsequent readjustment, are closely connected with the organization of the growing shoot, and it is therefore convenient to discuss the tissues in the young stem and branch before describing the process of occlusion in an older stem.

The method employed for studying occlusion was to take serial tangential sections from the base of the occluded branch outwards. The sections were made by means of a sliding microtome, stained with safranin and mounted in Canada balsam. Tracings of the annual rings were made from the sections, and from these it was possible to work out the changes in the surface of the trunk wood in the region immediately surrounding the pruned branch, and also to trace the gradual closing over of the cut surface of the branch by the occluding tissue.

2. TISSUE CONTINUITY BETWEEN BRANCH AND YOUNG STEM

The slender cylinder of wood in the base of a young branch is clothed with a cambium which is continuous with that of the main stem, both above and below the branch union. This continuity of the tissues from stem to branch is maintained on the lower side throughout the life of the branch, but on the upper side it is only the tissues of the bud stalk that form a continuous system with the wood of the stem. It will be seen in Pl. XXV, Fig. 1, that in the region of the crotch in a radial longitudinal section through the union, there is a conspicuous V-shaped zone of cells that are cut through in transverse section. This change in the direction of the cells above the branch is due to the reorientation of the cambial cells in the region of the crotch, which is associated with the propagation of cambial activity downwards from the buds of the branch and stem respectively. When the terminal bud begins to grow, cambial activity spreads down the stem and passes round the lateral bud, and this determines the orientation of the young developing tissues. When the lateral bud begins to grow, cambial activity spreads downwards from it on to the main stem. Near the main stem the cells on the top of the branch change their direction and turn to either side of the branch; passing round the branch, they are continued into that portion of the stem immediately below the point of entry. The branch is not firmly attached to the main stem on the upper side, and Pl. XXV, Fig. 2 shows an older branch which has been pulled downwards, and illustrates the fact that the wood of the branch is only continuous with the stem wood below the branch.

Text-fig. 1 is a sketch of the end of the extracted branch, and the shading



TEXT-FIG. 1. Sketch of the end of the extracted branch (the shading shows the direction of the cells).



TEXT-FIG. 2. Sketch of the socket in the stem from which the branch of Text-fig. 1 was extracted.

shows the direction of the cells. The lines on the top side of the branch pass in a horizontal direction until just before they reach the main stem, when they turn downwards and pass round the sides of the branch; at the lower side of the branch they curve inwards, and finally pass vertically downwards.

Text-fig. 2 is a sketch of the stem showing the socket from which the branch was extracted. The lines indicating the direction of the cells pass vertically down the stem until they reach the region immediately above the socket, when they diverge and pass round the socket; they do not unite immediately below the branch but pass vertically down the stem, outside the region that was in continuity with the branch wood.

Pl. XXV, Fig. 3, illustrates a tangential section through the region of the stem containing the socket, and shows the arrangement of the cells of the stem above and at the sides of the socket. The cells above and farthest removed from the socket are arranged more or less in vertical rows, but even in this region they show a tendency to be directed to either side of the socket; immediately above the socket the cells begin to curve to either side, and the cells on the top of the socket itself are arranged at right angles to those higher up the stem, and are parallel to the edge of the socket. In this example and in many other sections examined the majority of the cells are bending to the left of the socket, and, as will be pointed out later, this affects the development of the occluding tissue.

Pl. XXVI, Fig. 4, illustrates a tangential section through a branch and the surrounding stem wood, and shows the arrangement of the cells at the point of junction of the stem and branch wood. Above the branch, in the region of the crotch, the cells of the branch meet those of the stem, and the cells of both branch and stem turn in a horizontal direction in this region, passing round the sides of the branch and down the stem. Where the cells of the branch and stem join, above and at the sides of the branch the tissues are pushed outwards with the result that a lip is formed. This lip at the sides of the socket is illustrated in Pl. XXV, Fig. 2, and is indicated by ($\times$). The lip is formed in the region where the stem cells are contiguous to those of the branch but not continuous with them. Continued growth of the stem and branch causes a decrease in the area of the crotch which must be accompanied by a readjustment of the cambial cells in that region. In readjusting itself to this decrease in area the cambium bulges outwards, and this results in the formation of the lip of tissue. This may be still further intensified if the cambial cells continue to increase in number; further, any increase in the size of the cells in the region of the crotch may add to the size of the lip.

3. MACROSCOPIC EXAMINATION OF THE OCCLUSION OF PRUNING WOUNDS

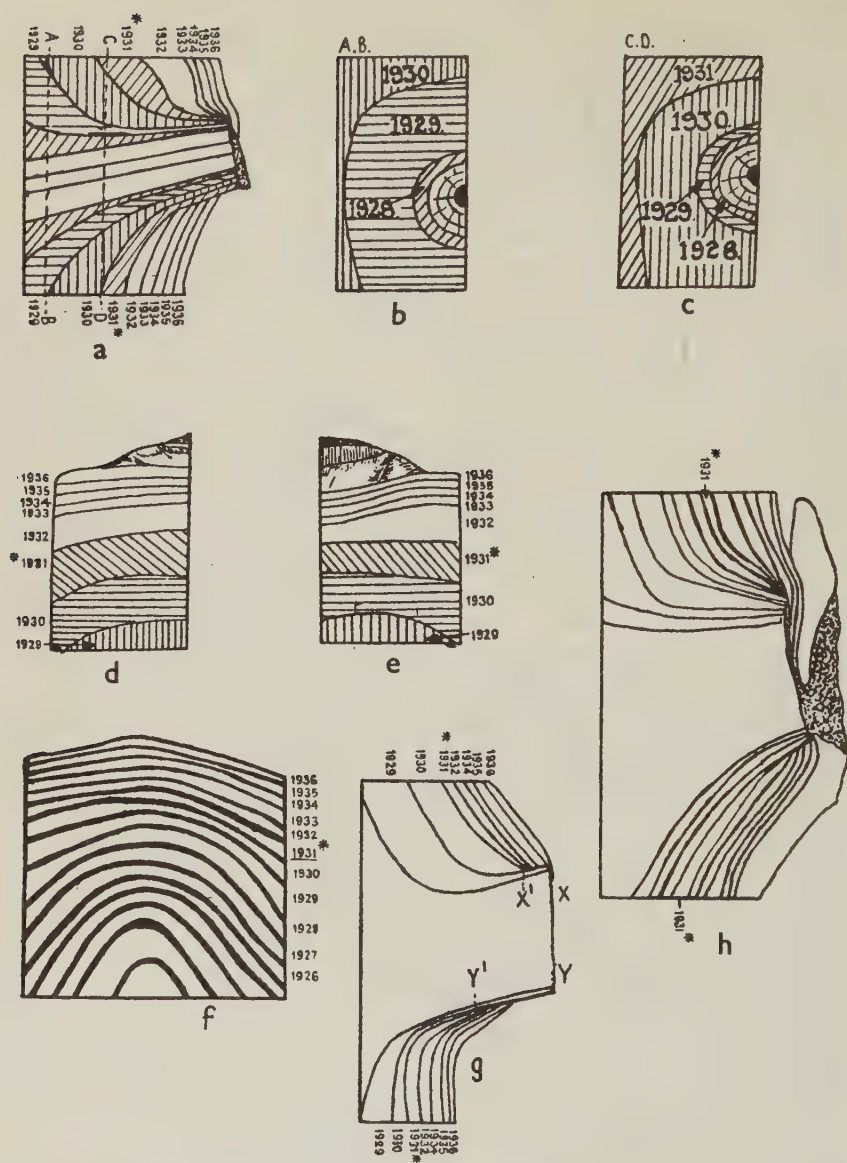
(a) *Observations on the occlusion of dead-pruned branches.*

In spruce occlusion only takes place by the activity of the cambium of the stem. The cut surface of the branch is gradually covered over, mainly from

the top, but also from the sides; very little, if any, occlusion takes place on the lower side of the branch. Observations have been carried out on a number of completely occluded dead-pruned branches, and in all cases examined the method of occlusion is the same. In timber produced after occlusion is complete, the grain is always distorted, and the nature of this distortion, which begins immediately after the death of the branch, is modified by pruning and the method of occlusion.

Grain of the wood before the death of the branch. The grain of the wood formed in the trunk before the death of the branch differs from that formed after the death of the branch. In a living branch the cambium of the branch is continuous with that of the trunk wood immediately below the branch, and is contiguous with the trunk cambium above and at the sides of the branch. It follows that cells cut off from the trunk cambium are contiguous with those cut off from the branch cambium above and at the sides of the branch. Thus, when the branch is alive, the annual rings of the branch correspond to those of the trunk, even if the tissues of the trunk do not form a continuous system with those of the branch, above and at the sides of the branch. This is shown in Text-figs. 3*a* and 4*c*. Fig. 3*a* illustrates a radial longitudinal section of a live-pruned branch, and the trunk wood immediately surrounding the branch. The annual rings are numbered, and the year when each annual ring was laid down is given; the annual rings laid down in the years 1928-31 are represented by the oblique, horizontal, vertical, and oblique shading respectively. The death of the branch took place in 1931; no further annual increment was added to the branch after this date, and there are no annual rings of the branch corresponding to those of the trunk laid down in the years 1932-6. Text-fig. 4*c* is a tracing of the annual rings taken from a transverse section through a dead-pruned branch, and the surrounding trunk wood. The annual rings are numbered, and it will be observed that the death of the branch took place after the wood of the ninth annual ring was laid down. The annual rings 1-9 of the trunk curve outwards and coincide with those of the branch; there are no annual rings of the branch corresponding with the annual rings 10-16 of the trunk. Tangential sections through the branch and the surrounding trunk wood before the death of the branch show the annual rings as concentric rings that gradually become smaller in diameter from the base of the branch outwards along the branch. This is shown in Text-fig. 3*b* and Text-fig. 3*c* which are taken in the positions indicated in Fig. 3*a*.

Before the death of the branch, the annual rings of the trunk wood immediately above the branch curve outwards. This is shown in Text-fig. 4*d*, which is a tracing of the annual rings taken from a transverse section of the trunk wood immediately above the branch shown in transverse section in Text-fig. 4*c*. The position of the branch is indicated by the dotted lines, and it will be observed that the annual rings 1-7 bulge outwards immediately above the branch. The annual rings 8 and 9 are practically straight, and this is probably due to the decrease in the activity of the branch. The grain of



TEXT-FIG. 3. All figures are tracings of annual ring limits in *Picea excelsa*. *a*. In a radial longitudinal section through a live-pruned branch. *b* and *c*. Tangential longitudinal sections through the branch union figured in *a*, in planes AB and CD respectively. *d* and *e*. Transverse sections of trunk wood immediately above, (*d*), and below, (*e*), branch shown in *a*. *f*. Transverse section of trunk wood immediately below a live-pruned branch in which cambium only died back a short distance. No distortion of annual rings. *g*. Radial longitudinal section through a live-pruned branch. Die-back at top and bottom of the branch indicated by xx' and yy' respectively. *h*. Radial longitudinal section of live-pruned branch showing occlusion is mainly from above. (Pruned in 1931.)

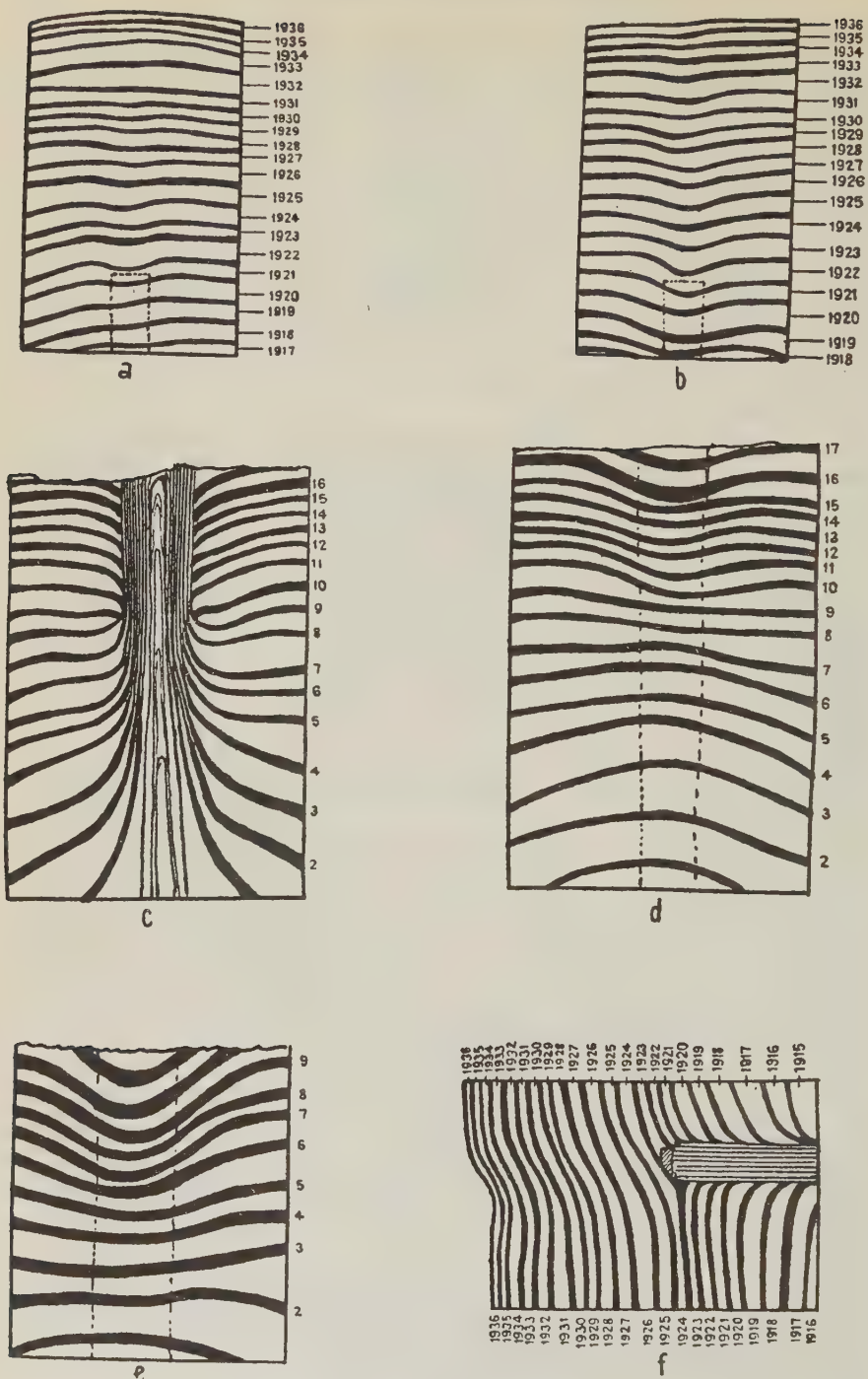
the trunk wood immediately below a dead-pruned branch is shown in Text-fig. 4e, which is a tracing of the annual rings from a transverse section of the trunk wood immediately below a dead-pruned branch. The annual rings 1 and 2 are those laid down before the death of the branch took place; these illustrate that the annual rings of the trunk wood immediately below the branch curve outwards. The position of the branch is indicated by the dotted lines, and it will be observed that the second annual ring shows a tendency to curve inwards immediately below the branch; this indicates that the activity of the branch is decreasing. The annual rings 3-9 are those laid down after the death of the branch, and show that there is a curving inwards of the annual rings immediately below the branch. It should be observed that the incurving of the annual rings becomes more pronounced as each successive annual increment is laid down.

Grain of the trunk wood after the death of the branch. The death of the branch results in a change in the surface of the trunk wood surrounding the branch, the main characteristics of which are, firstly, a slight depression above the branch that separates two areas of maximum growth occurring above and also at the sides of the branch, and secondly, a V-shaped depression below the branch. These characteristics of the trunk wood surrounding the dead branch are shown in Text-fig. 5 which illustrates a tangential section taken before the occluding tissue had reached the cut surface of the branch. The slight depression above the branch, the two areas of maximum growth, and the deep V-shaped depression below the branch are indicated. It was possible to distinguish the early and late wood of each ring, and these are represented by different shades of the same type of shading, light for the early wood and dark for the late wood.

In the same figure are seen sections in various planes through the wood shown in surface view. At E.F. is seen the slight depression (a) above the branch bordered on either side by the two areas of maximum growth L and L'. Text-figs. 4d and 4a are tracings of the annual rings from transverse sections of the trunk wood above dead-pruned branches, and show that the distortion of the grain caused by the two areas of maximum growth increases from the base of the branch outwards until occlusion of the cut surface of the branch is complete; from this point outwards the distortion is gradually decreased, due to the increased activity of the tissues below and at the sides of the two areas of maximum growth. In Text-fig. 4a it will be observed that straight-grained timber is produced by the annual rings laid down from 1933 to 1936.

The V-shaped depression (b) is shown in Text-fig. 5, section AB; the depth of the depression is equivalent to the width of the annual rings laid down in 1919 and 1920.

Text-figs. 4e and 4b are tracings of the annual rings from transverse sections of the trunk wood immediately below dead-pruned branches, and show that the distortion of the grain below the branch, caused by the slow rate of radial growth in that region, becomes more pronounced from the base of the branch



TEXT-FIG 4. (description at foot of p. 689).

outwards until occlusion of the cut surface is complete, and is then gradually decreased.

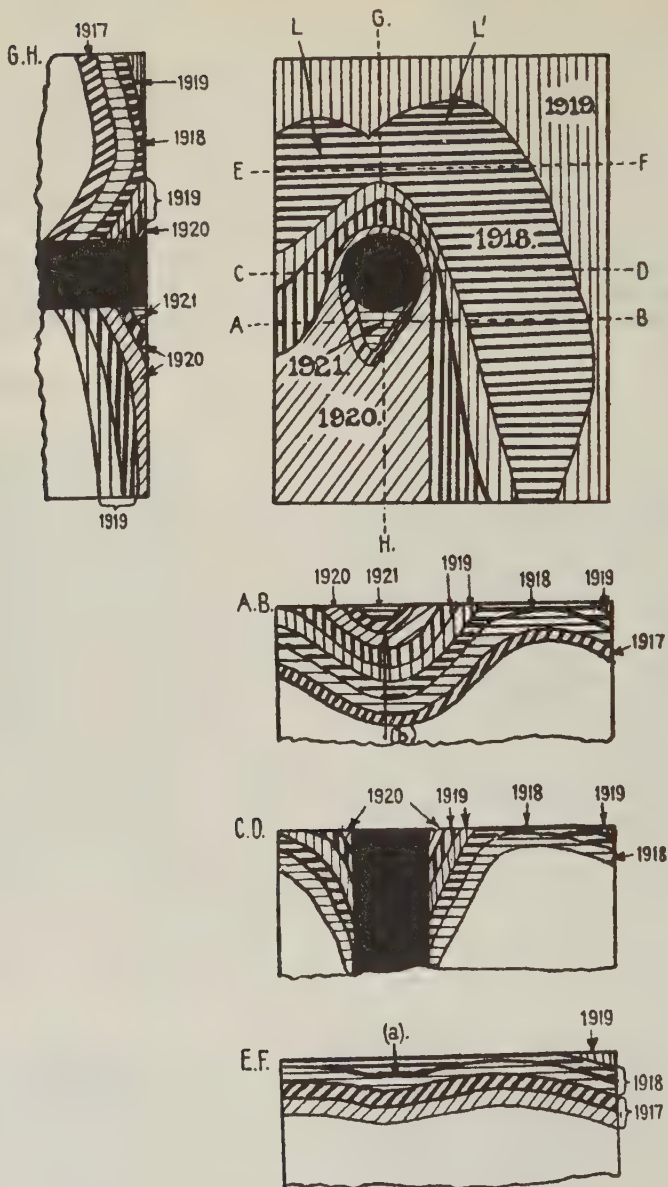
Comparison of Text-figs. 4*a* and 4*b* shows that the distortion of the grain is more pronounced below the branch than above the branch; also the distortion below persists for a longer period of time than that above the branch. When a branch dies, the annual growth rings of the trunk are discontinuous with the branch and curve inwards in the region of the branch, forming a slight depression which completely surrounds the branch (Curtis, 1906). This depression is shown in sections C.D. and G.H., Text-fig. 5. Section G.H. also shows that the occluding tissue above the branch is in advance of that below the branch, and this results in a distortion of the grain of the timber formed after complete occlusion of the branch.

Text-fig. 4*f* shows that this type of distortion is still considerable twelve years after complete occlusion.

(*b*) *Observations on the occlusion of live-pruned branches.*

When a live branch is pruned, the cambium of the stump of the branch dies back or dries out a short distance from the cut surface of the branch, but does not as a rule die back to its point of junction with the cambium of the trunk, as in the case of a dead branch. Thus in a live-pruned branch the occluding tissue is derived from both the cambium of the trunk and from the cambium of the stump of the branch, so that the method of occlusion differs from that of the dead-pruned branch. The extent to which the cambium of the stump of the branch dies back is shown in Text-fig. 3*g*, which is a tracing of the annual rings from a radial longitudinal section through the stump of the branch and the surrounding trunk wood; the cambium of the branch dies back a greater distance at the bottom than at the top, as is indicated by the distances YY' and XX' respectively. The death or drying out of the cambium results in the formation of a resinous layer completely covering that portion of the stump of the branch where the cambium has died. The occluding tissue gradually closes over the cut surface of the branch, mainly from the

TEXT-FIG. 4. *a*. Tracing of the annual rings from a transverse section immediately above a completely occluded dead-pruned branch of *Picea excelsa*. Complete occlusion took place in 1921, and maximum distortion of the grain is shown by the annual ring laid down in that year. *b*. Tracing of the annual rings from a transverse section immediately below a completely occluded dead-pruned branch. Complete occlusion took place in 1922, and maximum distortion of the grain is shown by the annual ring laid down in that year. *c*. Tracing of the annual rings from a transverse section through a dead-pruned branch. *d*. Tracing of the annual rings from a transverse section of the trunk wood immediately above a dead-pruned branch. Death of the branch took place after the 9th annual ring was laid down. Annual rings 10-17 show that the distortion of the grain is increasing. *e*. Tracing of the annual rings from a transverse section of the trunk wood immediately below a dead-pruned branch, after the death of the branch the annual rings curve inwards towards the centre of the tree. *f*. Tracing of the annual rings from a radial longitudinal section through a completely occluded dead-pruned branch, the occluding tissue laid down in 1924 below the branch, is in vertical line with the annual rings laid down above the branch in the two previous years.



TEXT-FIG. 5. Tracing of a tangential longitudinal section through the trunk in the region of a completely occluded dead branch of *Picea excelsa*. The wood of different annual rings is indicated by lines in different directions, thin lines for early wood, thick for late wood. AB, CD, EF, and GH are sections in various planes through the same trunk, the plane of section being indicated.

top, and also from the sides of the stump, as in the case of the dead-pruned branch; very little, if any, occlusion takes place from the lower side of the stump. This is illustrated in Text-fig. 3*h*. Observations have been made on more than thirty live-pruned branches, and in all cases the method of occlusion was the same.

The effect of live-pruning on the grain of the trunk wood. It was only possible to observe the initial effects of pruning, since completely occluded material was not available. There is very little distortion of the grain of the wood when a live branch is pruned; this is associated with the fact that in the live-pruned branch the base of the branch is completely surrounded with a living cambium. In the dead branch the death of the cambium causes a groove in the wood above the branch, and a deep V-shaped depression below the branch, but in the live-pruned branch the cambium in these regions remains alive and the groove and depression are not formed. In a few cases of live-pruning there is a slight depression in the trunk wood below the branch, which is associated with the dying back of the cambium for a short distance down the main stem below the branch. The amount of distortion of the wood of the stem depends upon the extent to which the cambium of the stump of the branch dies back. Another difference between the wood surrounding a live-pruned branch and that surrounding a dead-pruned branch is that the annual rings of the wood surrounding a live-pruned branch do not curve inwards towards the centre of the tree, as is the case in a dead-pruned branch. This is due to the fact that the branch cambium remains alive at the base of a live-pruned branch, with the result that the rings of the occluding tissue curve outwards along the branch. Text-fig. 3*a* is a tracing of the annual rings from a radial longitudinal section through a live-pruned branch and the surrounding trunk wood, and shows the curving outwards of the annual rings along the branch. The grain of the trunk wood above a live-pruned branch is shown in Text-fig. 3*d*, which is a tracing of the annual rings from a transverse section through the trunk wood immediately above a live-pruned branch; the annual rings are practically straight, and there is no incurving of the annual rings towards the centre of the tree as in the case of the annual rings of the trunk wood immediately above a dead-pruned branch. Text-fig. 3*e* is a tracing of the annual rings from a transverse section of the trunk wood immediately below a live-pruned branch. The years when the annual rings were laid down are given; the branch was pruned in 1931, and in this case the cambium of the branch died back on the lower side on to the main stem. The extent of the 'die-back' is shown in Text-fig. 3*g* by the distance xy' . Before the branch was pruned the annual rings of the trunk wood below the branch curved slightly outwards, and after pruning the annual rings immediately below the branch curve slightly inwards towards the centre of the tree. The distortion of the grain caused by this slight depression is very slight as compared with that in the trunk wood below a dead-pruned branch. This may be seen by comparing Text-fig. 4*e* with Text-fig. 3*e*. Text-fig. 3*f* is a tracing of the annual

rings from a transverse section of the trunk wood immediately below a live-pruned branch, in which the cambium of the branch has died back only a short distance from the cut surface. In this case the annual rings laid down after pruning curve outwards in the normal manner and there is no distortion of the grain of the wood (the branch was pruned in 1931). As there is less 'die-back' of the cambium with live pruning, there is a smaller area to be occluded. Consequently, healing tends to be more rapid, and owing to the absence of any groove below the branch, there is less distortion.

4. OBSERVATIONS ON THE BREAKAGE OF SPRUCE PIT-PROPS

Permission was obtained from the Leeds Fireclay Co. Ltd. to carry out the observations at Tong Lane Mine, nr. Bradford. The observations were made on the main return road, where the replacement of broken props was an expensive and serious matter, in fact so much so that the props and bars are now being replaced by brick side-walls, and steel girders. Observations were also carried out on several of the 'tramming' roads. The dimensions of the props examined varied from 4 to 5 ft. in length, and from 4 to 5 in. in diameter. In all the cases examined breakage had taken place at the whorl of knots, see Pl. XXVII, Fig. 5; in the majority of cases the break was confined to this region only, but in a few cases the break had begun at a knot and spread obliquely across the prop above the whorl of knots. It was also observed that straight, smooth, clean (free from knots) props had withstood the pressure as also had sawn square (4 in. $\times$ 4 in.) 'legs' free from knots. The observations on the position of breakage point out that any defect in the form of a whorl of knots in timber used for mining purposes is a serious source of weakness. It is evident from the previous observations on the arrangement of the tissues of a young stem and branch that this weakness at the whorl of knots is associated with the arrangement of the tissues at the point of junction of the branches with the main stem. The wood of the branch is contiguous with that of the main stem both above and at the sides of the branch, and the wood of the branch is only continuous with that of the main stem below the branch; hence at the whorl of knots the area of vertically continuous wood subjected to pressure is considerably reduced. As a result of this, the pressure per unit area in this region is increased beyond the breaking point. If, therefore, the area of vertically continuous wood can be increased at such a point, e.g. by pruning (when a complete continuous vertical cylinder of wood is produced), then the pressure per unit area at the position of the whorl of knots is decreased as is also the tendency to break at this point.

5. MICROSCOPIC EXAMINATION OF THE OCCLUSION OF PRUNING WOUNDS

In the spruce the occluding tissue is derived solely from the activity of the cambium, and the arrangement of the cells has an important bearing on the

process of occlusion, as the cells of the wood and bast have approximately the same shape as the cambial cells which produce them. In the live branch, as the cambial cells of the main stem above and at the side of the branch do not form a continuous system with those of the branch, they are little affected by the drying out of the wound surface. This drying effect tends to follow the course of the tissues and thus to reach the main stem below the branch. On the upper side of the branch the cambial cells themselves are turning at right angles to the branch axis, and even if several cells dry out and die, this injury will only extend a very short distance on to the trunk. Thus, after pruning a live branch, the drying out of this cambium is more extensive on the trunk beneath the branch. When a branch dies its cambium as a rule dies back to its point of junction with that of the trunk, the effect spreads to the trunk cambium and in an area around the base of the branch, and in a V-shaped area below the branch no living cambium is left.

It follows that in the dead-pruned branch the occluding tissue must be derived from the activity of the trunk cambium, but in the live-pruned branch the occluding tissue is derived partly from the activity of the trunk cambium and partly from the activity of the cambium surrounding the base of the branch. This absence of a living cambium at the base of a dead-pruned branch greatly retards the occlusion of the branch, since before occlusion can take place the cambial cells of the trunk surrounding the area have to grow to fill the gap at the base of the branch. The spreading of the trunk cambium over this area at the base of the branch is accompanied by a reduction in radial growth, so that the annual rings of the trunk dip inwards in the immediate vicinity of the branch, and this continues until the area at the base of the branch has been covered with living cambium. These areas at the base of the branch and on the trunk immediately below the branch cause distortion of the grain of the trunk wood, and the greater the area to be covered, the greater the distortion of the grain. In some cases two to three years are needed for the trunk cambium to spread over this area on the trunk below the branch, whereas the area above and at the sides of the branch is covered with a living cambium in one year after the branch has died. Consequently, the development of the occluding tissue above the branch is in advance of that below the branch, but once there is a complete cambial sheath surrounding the base of the branch, occlusion of the stump of the branch is governed by the rate of radial growth of the trunk. Pl. XXVI, Fig. 6, illustrates a tangential section through a dead-pruned branch and the surrounding trunk wood, and shows that occlusion of the branch takes place mainly from the top and sides of the branch. It will be observed that in this case the occluding tissue is advancing obliquely over the cut surface of the branch, and that the occluding tissue on the left is slightly in advance of that on the right of the branch. In many branches this lip of occluding tissue is more oblique than in the example shown and appears to be associated with the manner in which the cells of the trunk cambium diverge and pass round the branch; in this example it will be

seen that the majority of the cells pass to the left, that is, there is a right-handed spiral grain in the wood above the branch (Champion, 1925).

In a live-pruned branch occlusion begins immediately the cambium becomes active, as the base of the branch is completely surrounded by a living cambium, and there is therefore no time-lag except in a few cases in which the cambium has dried out for a short distance down the trunk below the branch. Pl. XXVII, Figs. 7 and 8, illustrate radial longitudinal sections through live-pruned branches and the surrounding trunk wood, and show that the cambium only dies back a short distance from the cut surface of the branch. Fig. 7 also shows occlusion taking place from the top of the branch, and it will be observed that occlusion of the cut surface of the branch began in the first growing season after pruning and was practically complete six years later. A slight resin pocket was formed between the occluding tissue and the cut surface of the branch, but the resin was removed in sectioning. The photograph also illustrates the abundant development of vertical resin canals in the trunk after pruning. Radial resin canals in the fusiform rays are also abundant in the occluding tissue over the cut surface of the branch, but can only be seen in tangential section.

The occluding tissue is derived mainly from the cambium of the stump of the branch, which remains alive after pruning, but occlusion is also assisted by the general downward movement of the cambium as a whole, after the occluding tissue has reached the cut surface of the branch. This downward movement of the cambium is indicated by the direction of the rays before and after pruning. The direction of the rays before pruning is obliquely upwards and outwards, but immediately after pruning the direction becomes practically horizontal, and finally is slightly downwards. Priestley (1936) points out that in the normal stem the rays represent rigid bars of vacuolated tissue traversing a semi-fluid layer of meristematic cambium; this change in the direction of the rays after pruning is due to an alteration of the forces holding the cambial cells in a fixed position. The fact that the occluding tissue in the case of a live-pruned branch is derived from the cambium of the branch is evidence for the lateral stimulation of the cambium, as the cambial cells of the trunk are not continuous with those of the stump of the branch. After occlusion is complete, there is a gradual reorientation of the cambial cells, until they become regularly arranged as in the normal stem.

6. RESIN PRODUCTION IN PRUNED BRANCHES

In both live- and dead-pruned branches resin is only present in that portion of the branch which is not in symplastic continuity with the wood of the main stem. The black 'loose' knot of timber is formed by the complete occlusion of a dead-pruned branch, and resin is only present in that portion of the knot which is loose. The length of knot which is loose depends upon the length of the 'snag' left after the dead branch was either broken off

or pruned. Resin is not produced in a dead branch, nor is resin produced in that portion of the snag of a dead-pruned branch which is not buried by the occluding tissue. Samples of living, moribund, and dead branches were collected and tested for starch, oil, and resin.

The ray cells of the live branch were packed with starch; those of the moribund branch contained very little starch, and there was a complete absence of starch from the ray cells of the dead branch. Sections were stained with Sudan III, and in all cases there was a complete absence of oil or fat in the wood. Sections were placed in a saturated solution of copper acetate, and left for 1-3 weeks; in all cases there was a complete absence of resin.

The complete absence of starch, oil, or resin in the dead branch, and the small amount of starch in the ray cells of the moribund branch, is evidence for the complete respiration of all available food reserve before the death of the branch takes place. Swarbrick (1926) records that the production of 'wound gum', which blocks the tissues of a pruned branch, is associated with the disappearance of starch from the parenchyma cells.

From the observations it is obvious that there can be no production of resin from starch in the case of the dead branch, but when a live branch is pruned there is a small amount of resin or wound gum produced near the cut surface; this production of wound gum may be due to a drying out of the tissues at the cut surface of the branch resulting in a change in the metabolism of the parenchyma cells in that region, and a conversion of starch into wound gum.

All the resin in a dead-pruned branch, and most of the resin in a live-pruned branch, is produced by the occluding tissue. There is an influx of wound gum into the 'snag' of the dead-pruned branch from the occluding tissue surrounding the snag. The resin is not uniformly distributed throughout the 'snag' of the dead-pruned branch; there is a gradual reduction in the amount of resin in the tissue from the periphery to the centre, that is, there is a penetration of wound gum from the occluding tissue into the 'snag' of the branch as is shown by the resin gradient. Further evidence for the influx of wound gum from the occluding tissue is the increase in the number of resin canals in the occluding tissue as compared with the normal tissue, and the presence of resin in the cells of the occluding tissue surrounding the snag of the branch, see Pl. XXVI, Fig. 6. In the case of the live-pruned branch a covering of resin is produced on the outside of the branch in the region where the cambial sheath has died or dried out; no such resinous covering is produced in the case of a dead branch. From the evidence it is apparent that there is no blocking of the cut surface of a dead-pruned branch, and there is no effective blocking of the branch until occlusion begins, that is, not until the trunk cambium is reactivated in the growing season. In the live-pruned branch, however, a certain amount of blocking of the cut surface of the branch takes place, and the branch is less open to the danger of fungal infection, a point in favour of live-pruning. Another important point in

favour of live-pruning is that there is no production of the black 'loose' knot such as is associated with the occlusion and influx of wound gum into the snag of a dead-pruned branch.

7. DISCUSSION

In spruce the dead branches persist for many years even in well-stocked plantations, and close planting is not effective in producing clean timber. Dead branches persist over a long period of the life of the tree and, consequently, knots are formed for a considerable period; in fact, for a period equivalent to the length of any rotation now commercially possible. If clean timber is to be produced on a commercial rotation, artificial pruning is essential. It has been the practice to prune dead branches only, but in spruce this has not been very successful owing to the slow rate of occlusion of the dead-pruned branches, which often extends over 8–10 years. The time taken for complete occlusion of live-pruned branches appears to be considerably less (5–7 years); but only a few completely occluded live-pruned branches were available for examination, and the results require further verification. Apart from factors such as diameter of branch, length of snag to be covered, &c., the rate of healing depends mainly on the rate of radial growth in the trunk, but also on the extent to which the cambium of the branch dies back after pruning. When a branch dies, the cambium of the branch dies back to its point of junction with the cambium of the trunk, and this 'die-back' spreads for a short distance down the trunk below the branch. When a live branch is pruned, the cambium of the branch only dies back a short distance from the cut surface of the branch, and consequently occlusion is much more rapid. This difference in the extent to which the cambium dies back in live- and dead-pruned branches accounts very largely for the difference in the time taken for complete occlusion in the two cases. The amount of 'die-back' of the cambium is very important and is probably connected with the season in which pruning is carried out; further research on this point is desirable. It is obviously important with both live and dead branches to prune as close to the main stem as possible, and the method of occlusion points to the importance of not leaving small snags at the bottom of the branch such as often occurs with the use of the pole saw. The direction of the cut should be downwards and inwards, rather than downwards and outwards. Live-pruning causes less distortion of the grain of the timber than dead-pruning, and the snag is more rapidly occluded. On the whole, the evidence is in the favour of live-pruning.

Mayer-Wegelin (1936), however, states that live-pruning in spruce is not advisable as it increases the risk of fungal infection. The material available for this investigation was not sufficient for definite refutation of this statement, but no fungal infection of live-pruned branches was observed.

Both Curtis (1936) and Hawley and Clapp (1935), working on conifers in general, state that live-pruning does not lead to fungal infection. From the

observations on 'resin production in pruned branches' it would appear that there is no effective blocking of the cut surface of a dead-pruned branch, and the entire area of the cut surface of the branch is open to fungal infection.

8. SUMMARY

1. The arrangement of the tissues of the young stem and branch is described. The bud stalk is continuous with the tissues of the stem above and below, but after the growth of the bud, the cambial cells in the region of the crotch become reorientated so that the branch tissues all turn downwards and are only continuous with the stem wood below the branch.

2. The spread of cambial activity down the branch stimulates the growth in a V-shaped area on the stem below the branch. As the growth of the branch slows down a depression is formed in the trunk wood below the branch.

3. Owing to the orientation of the cells of the branch, 'die-back' after pruning is most severe on the lower side of the branch in both live- and dead-pruned branches, but in the live-pruned branch the cambium only dies back for a comparatively short distance from the cut surface.

4. Live-pruned branches occlude more rapidly and cause less distortion of the grain than dead-pruned branches, and no evidence was found of any greater liability to decay in the former.

5. The black 'loose' knot of timber is associated with the occlusion and influx of 'wound gum' from the occluding tissue into the snag of a dead-pruned branch.

6. Breakage of pit-props takes place at a whorl of knots, since in this region the area of vertically continuous wood is reduced and the pressure per unit area increased.

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EXPLANATION OF PLATES XXV TO XXVII

Illustrating Mr. A. Paterson's paper on 'The Occlusion of Pruning Wounds in Norway Spruce (*Picea excelsa*)'

PLATE XXV

Fig. 1. Photograph of a longitudinal section through a two-year-old stem of *Picea excelsa* which has developed a lateral shoot. (a) pith domes, (b) continuity of branch and stem wood,

(c) V-shaped zone of cells cut through in transverse section (stem and branch wood contiguous).

Fig. 2. *Picea excelsa*. Photograph of a dislodged branch, illustrating the continuity of the branch and stem wood below the branch only. x, lip of trunk tissue above and at the sides of the branch.

Fig. 3. *Picea excelsa*. Photograph of a tangential section through the region of the stem containing the socket. The majority of the cells are bending to the left of the socket and the tracheids at the edge of the socket are arranged with their long axes parallel to the edge of the socket.

PLATE XXVI

Fig. 4. *Picea excelsa*. Photograph of a tangential section through a branch and the surrounding stem wood. (a) The region in the crotch where the cells of the stem and branch are contiguous; the majority of the cells of both the stem and branch in this region are bending to the left.

Fig. 6. Photograph of a tangential section through a dead-pruned branch, and the surrounding trunk wood, showing the arrangement of the tissues, and the oblique advance of the occluding tissue over the cut surface of the branch, and also the resin in the cells of the occluding tissue immediately surrounding the branch.

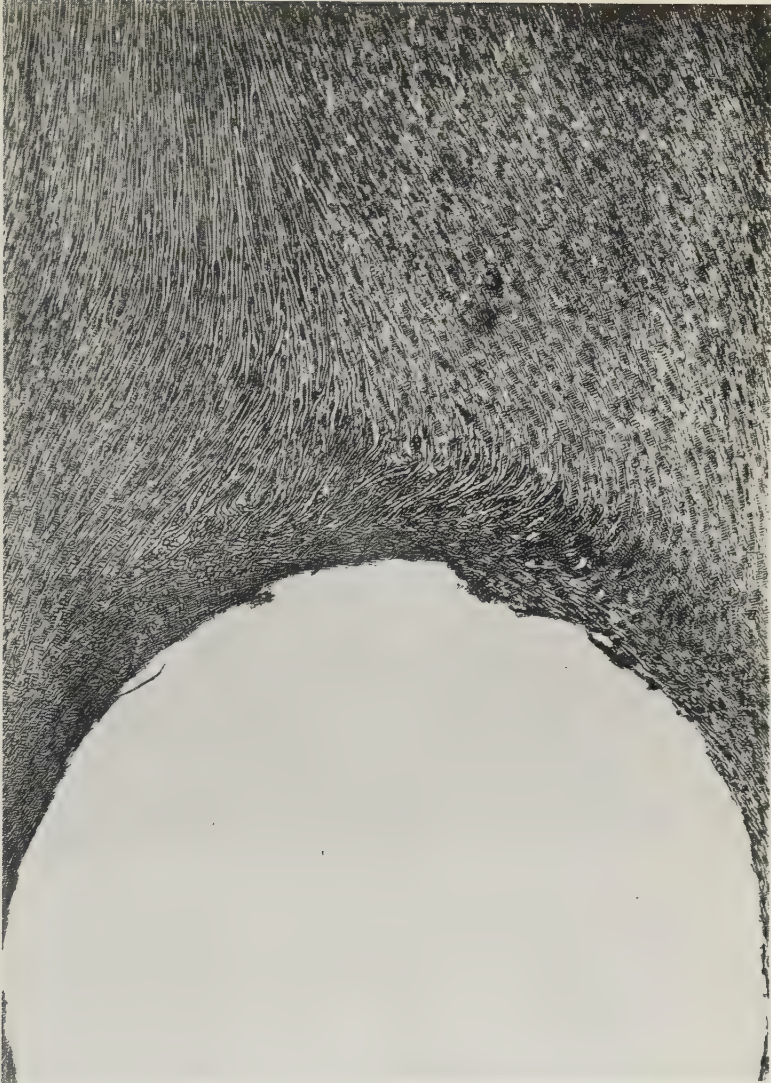
PLATE XXVII

Fig. 5. Photograph of a broken pit prop. (a) Knots, (b) sockets from which the knots were extracted when breakage took place, (c) area of vertically continuous wood.

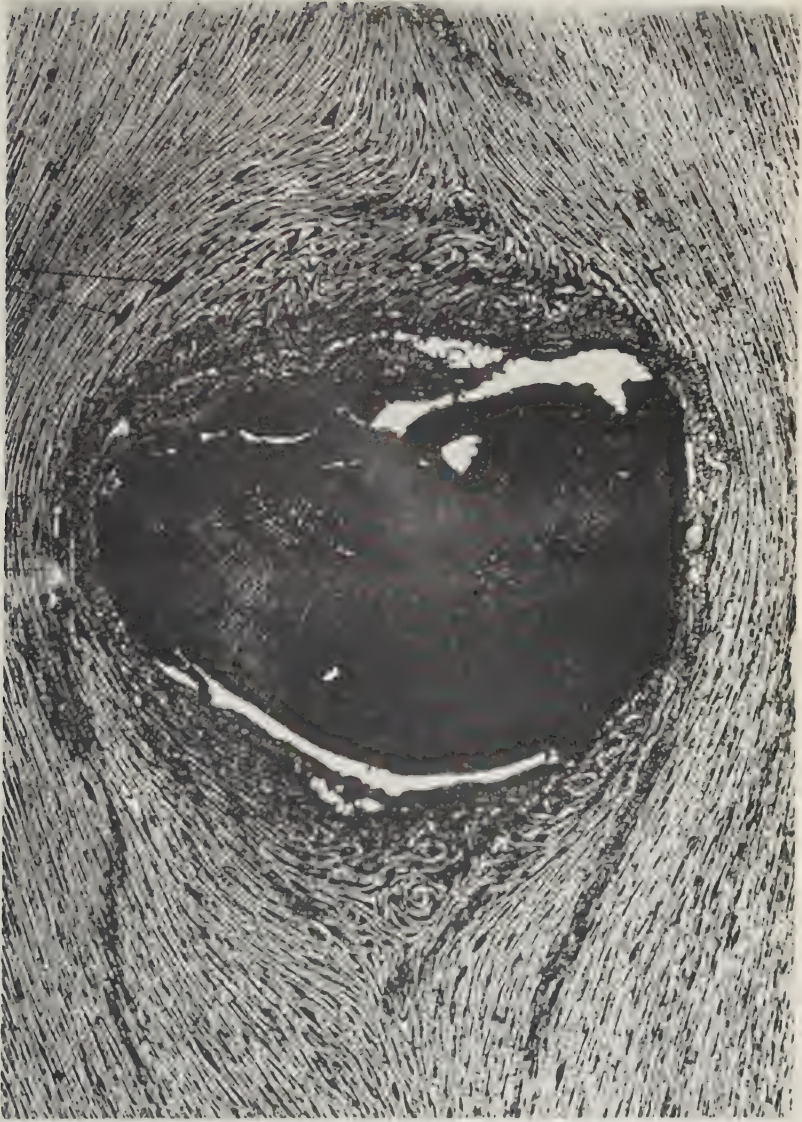
Fig. 7. *Picea excelsa*. Photograph of a radial longitudinal section through a live-pruned branch, and the surrounding trunk wood, which shows the abundant development of resin canals in the occluding tissue, and the change in the direction of the rays after pruning. (a) Branch, (b) resin pocket (resin removed during sectioning), (c) lip of occluding tissue xx' and yy', extent of the death of the cambium at the top and bottom of the branch respectively.

Fig. 8. *Picea excelsa*. Photograph of a radial longitudinal section through a live-pruned branch and the surrounding trunk wood. Note the change in the direction of the rays after pruning of the branch, and the abundant development of resin canals in the occluding tissue. The extent of the die-back of the branch cambium at the top of the branch is indicated by the distance xx'.





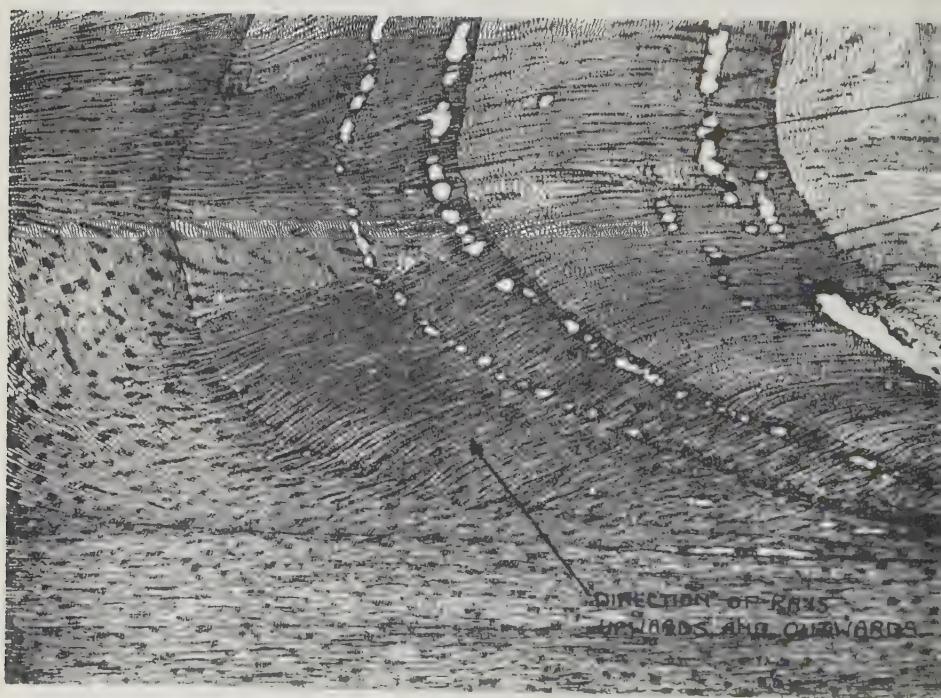




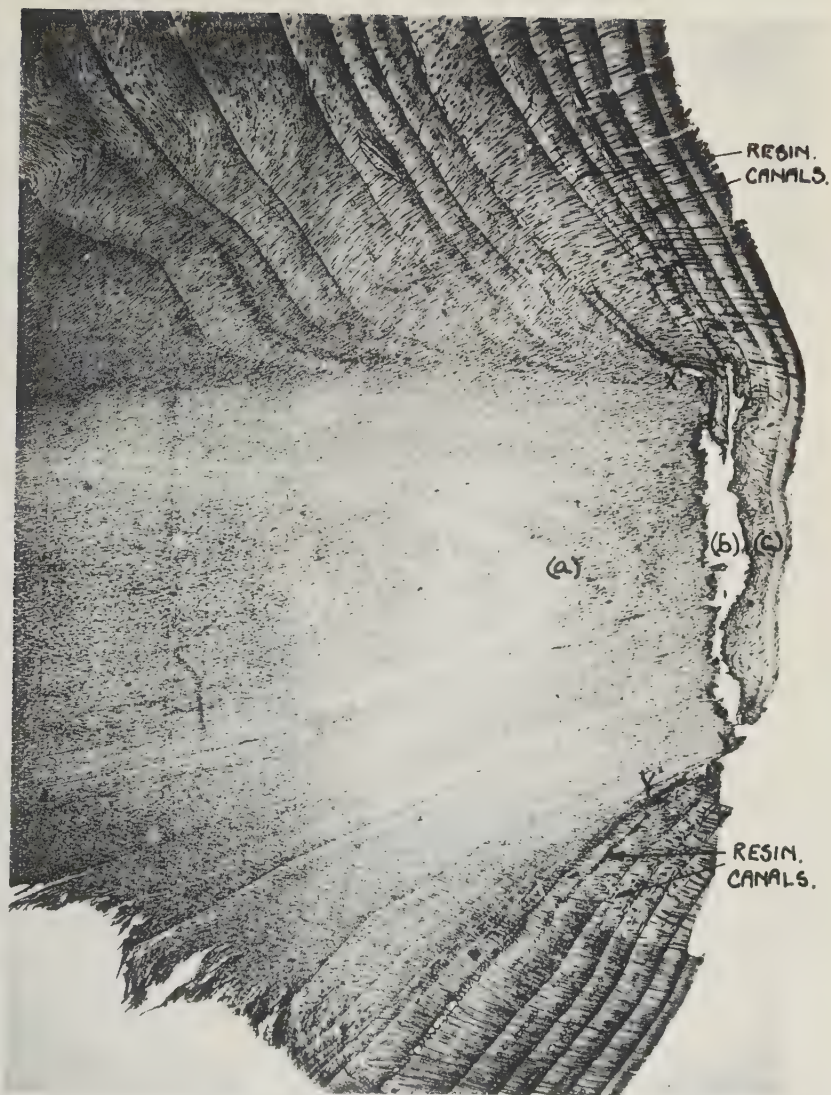
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5



PATERSON — OCCLUSION OF PRUNING WOUNDS.



Biological Technique for the Evaluation of Fungicides

I. The Evaluation of Seed Disinfectants for the Control of Helminthosporium Disease of Oats

BY

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With Plate XXVIII

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INTRODUCTION

THE increasingly widespread recommendation and use of proprietary fungicides as seed dressings for the prevention of seed-borne diseases, particularly those of cereal crops, has led to an immediate demand for accurate knowledge regarding their efficiency. The information available regarding their active constituents and methods of manufacture is insufficient for their present recommendation on these grounds alone, with the result that their value can only be satisfactorily measured by the biological method of testing their efficiency for disease control. It is desirable that such knowledge should be made available as speedily as possible and any technical improvement which assists towards the development of more rapid and accurate testing methods will serve towards this end. The present paper deals with the biological technique available for the assessment of the values of seed disinfectants for the control of Helminthosporium disease of oats, and the results obtained from an investigation of the problem over the three-year period 1935-7 are presented.

The established method of evaluating seed disinfectants for the control of the primary phase of Helminthosporium disease is by laying down field plots sown with disinfected grain and adjudging the results by making counts of the diseased seedlings produced. This method is desirable in that the work

is carried out under conditions closely allied to those obtaining in farm practice whereby the results obtained are at once applicable to the farm. It has, however, the drawback of being slow, and on account of the close relationship between the occurrence of this disease in epidemic form and the seasonal conditions prevailing it is usually necessary to extend the trials over a period of years before reliable data regarding the efficiency of the fungicides under test can be obtained. It was because of this that the writer commenced an investigation in 1934 dealing with the epidemiology of this disease, the results of which have already been published (Muskett, 1937) and have led to the development of a pot-culture technique for the testing of seed disinfectants whereby the occurrence of the disease in epidemic form can be relied upon and the fungicides submitted to rigorous testing at will. The results of the application of this technique to routine testing are given. In addition, the development of laboratory technique whereby tests can be made without recourse to the growing of the seedlings in soil has been investigated. The results of this work are also described, together with the comparative results obtained by employing laboratory, pot, and field methods over the three-year period under review.

LABORATORY METHOD

If oat grains selected at random from a sample of seed infected with *Helminthosporium Avenae* Eid. are incubated under moist conditions at a suitable temperature, then those which are infected will be revealed by the growth of the fungus from the grain. An opportunity is thereby afforded for the estimation of the degree of infection in any given seed sample, but, unfortunately, the rapid diagnosis of the fungus by mycelial characters alone is difficult when it is growing alongside species of *Alternaria* and *Cladosporium*, &c., and almost impossible in the case of routine work where the examination of large numbers of grains is necessary and the work has to be speedily accomplished. Its recognition by the production of conidia is, however, comparatively simple, and conidial production has therefore been taken in this work as the criterion for the determination of an infected grain. Again, it is unfortunate that *H. Avenae* does not always produce conidia readily, with the result that this method of diagnosis, although very reliable, tends to an under-estimation of the amount of infection. The tendency towards conidial production has also been found to vary in different seed samples. Such being the case, anything which will encourage sporulation of the fungus will assist towards a more accurate assessment of infection.

Dillon Weston (1933, 1936) has shown that sporulation in artificial cultures of *H. Avenae* may be induced by exposing them to white light of high intensity, and an attempt has been made to determine whether this holds true for the fungus when growing on naturally infected oat grains. The source of light, as suggested by Dillon Weston (1933), was a Hanovia quartz mercury vapour artificial alpine sun-lamp (direct current, 220 volts). The grains were sub-

mitted to twenty minutes' irradiation at a distance of approximately 1 ft. (30.5 cm.) after having been incubated under moist conditions for three days at 22° C. Experiments with a large number of oat-seed samples have shown that there is a tendency for the fungus to sporulate more freely after exposure to such irradiation, and the observations of Dillon Weston regarding the more intense pigmentation of the mycelium after treatment has also been confirmed. The species of *Alternaria* commonly associated with seed oats was found to behave in a similar manner, irradiation leading to increased sporulation and more intense pigmentation. With regard to the increase in sporulation for *H. Avenae* the results obtained using different samples of seed were not consistent, the increases in percentage infection obtained after irradiation ranging from 0 to 20 per cent., although in no case did irradiation result in lowering the percentage infection recorded. It may therefore be deduced that irradiation of the grain during the period of incubation, while not always producing significant results, is of some assistance when examining by laboratory methods oat samples for infection with *H. Avenae*; the more profuse sporulation of the fungus obtained by irradiation, leading to greater ease of identification, is also helpful.

The main purpose of this investigation was not to determine the amount of infection of oat samples, but to find the use of the method in evaluating seed disinfectants. Therefore, as soon as the method had been elaborated it was put to the test by determining the amounts of viable infection present in disinfected samples of seed and comparing these with the amounts in similar samples which had not been disinfected. Preliminary experiments indicated that in the case of seed disinfectants, the worth of which had already been proved by other methods, the development of *H. Avenae* and, incidentally, all other fungi was completely suppressed, whereas for disinfectants which only give partial control of the disease the development of *H. Avenae* was but partially suppressed. It was therefore decided to introduce the laboratory method as routine practice, and the results obtained by its adoption over the three-year period are presented and discussed in a later section of this paper in relation to those obtained by the pot and field methods. The laboratory method as used at present is outlined below, and although the technique is open to further improvement, the present recommendations are based upon the result of considerable experimentation, particularly with regard to such points as the most suitable temperature for incubation, the optimum time for irradiation, the total incubation period necessary, the most suitable optical equipment for use in the examination of the grains, and the number of examinations necessary.

Details of method.

First day. Mix thoroughly the sample of seed under investigation and select at random 100 grains. Arrange them with equidistant spacing in Petri dishes, each of which has the bottom lined with two moistened filter-papers.

Pack the dishes in an open container, the mouth of which is afterwards closed with cloths saturated with water in order to prevent drying out, and place the container in an incubator set to give a constant temperature of approximately 22° C.

The Petri dishes used are of 9 cm. internal diameter, 15 seeds being counted into each. Five hundred seeds were used for each sample at first, but the number was later reduced to 100, which was found to give equally useful and reliable results. The filter-papers used just fit the dishes; they are placed dry in the lower half of each dish which is then almost filled with tap-water, the surplus water being drained off after the papers have become saturated.

Fourth day. Remove the dishes from the incubator and irradiate the seeds for 20 min., using a Hanovia quartz mercury vapour lamp operating at a distance of approximately 1 ft. (30.5 cm.) from the dishes. Repack the dishes in the container and replace in the incubator.

The lids of the dishes are removed during irradiation, the duration of their removal being insufficient to cause drying out if the filter-papers have been thoroughly saturated. According to Dillon Weston (1936), sporulation is induced by intense white visible light and not by the invisible ultra-violet rays. It is therefore probable that an alternative source of white light would be suitable, but as the Hanovia lamp was available and the method found convenient before Dillon Weston's later findings, no changes have been introduced.

Ninth day. Examine the seeds for the presence of conidia of *H. Avenae*. The shoots are first removed with a sharp scalpel in order to facilitate examination. The most suitable microscope used so far for this purpose is a Wide Field Bausch and Lomb Binocular model, the optical equipment selected being paired eyepieces (15×) used with paired objectives (4.0×) giving a magnification of 60×.

Only one examination has been found to be necessary. Repeated examinations after the ninth day may reveal one or two more infected grains, a result which is not significant for the present purpose. Examination before the ninth day is not necessary, and as the mature conidia are easily detached from the conidiophores, the less the dishes are handled the better. Conidiophore production only is not regarded as sufficient for diagnosis of infection—at least one conidium must be present. During examination the glume apices are first inspected, then the basal end of the grain, and finally the remainder of the grain surface. In no case has the spread of the fungus been observed from an infected grain to one which is disease free, although during the time taken for the test it is not uncommon for the fungus to grow out from an infected grain to a distance of a few millimetres on the filter-paper.

POT-CULTURE METHOD

The pot method depends for its efficiency upon raising oat seedlings to the stage where the length of the second leaf approximates to that of the first and

under conditions designed to produce the maximum incidence of the disease. The necessary technique was elaborated by Muskett (1937) in the course of an epidemiological study made during 1934-6. It was found that the disease in an easily recognizable form could be obtained at will by growing the seedlings in moist soil (50 per cent. saturation) in metal containers at a relatively low temperature. An open outdoor veranda erected along the wall of one side of the flat roof of the Agricultural Building was used for the purpose, the roof of the veranda being sloped at such an angle as to allow a maximum of light to reach the plants and projecting sufficiently to shelter them from rain. The moisture content of the soil was kept constant by regular watering on alternate days. The relatively low temperature conditions, averaging from 8° C. to 10° C. during the experimental period, may normally be obtained in Northern Ireland by sowing the seed about March 17. The use of a lower temperature than this favours the occurrence of the pre-emergence phase of the disease which is not so easily diagnosed, while the employment of higher temperature leads to a falling off in the incidence of the disease. The temperature employed has been found to encourage a maximum of the easily recognizable leaf-stripping phase. When the seedlings have grown to the stage referred to above, each is cut off at soil level and the extent of the disease is assessed by visual examination. Only those seedlings which show 'stripe' lesions on the first, second, or third leaves are counted as diseased. This method of disease assessment, besides being reliable, allows of the rapid examination of large numbers of seedlings.

During the study it was found that there is a tendency for some dust disinfectants to give less satisfactory results under conditions of high soil temperature and dryness, a finding which indicates the necessity for testing their efficiency under such conditions. Fortunately, these conditions also favour the disease, and they may normally be obtained in Northern Ireland by raising seedlings as outlined above in comparatively dry soil (25 per cent. saturation) during the late spring. The suitable mean temperature of from 12° C. to 14° C. during the experimental period may normally be obtained by sowing the seed in mid-May.

For the past three years the pot method has been employed in routine practice for the assessment of seed disinfectants used against *H. Avenae*, two tests being made each season. The results from the second test, in addition to recording the behaviour of the fungicide under conditions of dry soil at a high temperature, serve as a useful check upon those obtained by the first. An outline of the pot method in detail is as follows:

Details of the method.

Soil. As soil type does not appear to be an important factor, a medium loam of good quality is recommended for test purposes. The soil is air dried by spreading it on the floor of a heated greenhouse, sieved, and stored in galvanized iron bins until needed. When required, the necessary quantity is

spread out on a hard floor, sprinkled with water from a hose, and thoroughly mixed; this process is repeated until the required degree of moistness has been reached. After a little experience this can be judged with sufficient accuracy by feeling the soil texture with the hand. A sample of the prepared soil is bottled and put aside for moisture estimation.

Pots. Round tins 22 cm. diam. $\times$ 11.5 cm. high, such as are employed for the baking of cakes, are used as pots, ordinary flower-pots being unsuitable on account of their porous nature and the consequent difficulty of maintaining a constant soil moisture content. This size of tin is suitable for raising 100 seedlings to the desired stage of growth. The tins will last for at least three years if looked after carefully.

Sowing. The same quantity of soil is weighed into each pot, the quantity varying only in the case of different moisture contents. A decimal scale (sensitive to 1.0 gm.) is very suitable for these weighings. Each pot is filled to within about 1.5 in. (4.0 cm.) of the rim, the soil being lightly compressed with a wooden dumper and the weight rechecked before sowing. Sufficient of the soil is put aside for covering the seed after sowing. The even distribution of the 100 seeds sown in each pot is secured by sowing through holes, cut equidistantly in a disc of ply-wood made so as to fit neatly inside the pot. Before sowing, holes of equal depth are made to receive the seeds by the use of a small wooden punch designed to fit the holes in the disc. In order to avoid the rubbing of dust disinfectants from the seed, forceps are used for sowing. After sowing, the seeds are carefully covered with soil of the same moisture content as used for filling the pot, it being assured by weighing that the same amount of covering soil is used for each pot. The pots are then placed in their permanent quarters. (See Pl. XXVIII, Fig. 1.)

Number of seeds. The use of 500 seeds for each sample under investigation, i.e. five pots, has been adopted as the standard procedure in routine practice.

Care after sowing. The moisture content of the pots is kept constant by watering on alternate days. When watering, each pot is brought up to the weight registered immediately after sowing.

Temperature control. A self-recording thermometer is kept among the pots during the whole test period. The mean temperature for the test is assessed by averaging the temperatures recorded at two-hourly intervals during the whole trial period.

Duration of test. First test (moist soil and low temperature) approximately 40–50 days; second test (dry soil and high temperature) approximately 30–40 days.

FIELD METHOD

The field technique normally employed, which has been elaborated here, consists of the sowing of disinfected grain under field conditions and examining the seedlings for the presence of the disease when the symptoms have become fully apparent. Except for the fact that the work is carried out under less controlled conditions the mode of procedure resembles that adopted for

the pot method. The technique here described has been arrived at after considerable investigation made necessary on account of the various factors, often unforeseen and sometimes unavoidable, which may interfere with the accuracy of a field trial. Erratic damage by pests such as birds, rabbits, and hares was overcome in 1936 by making the plots of such a size and shape as to allow of their being covered with wire-netting fitted immediately after sowing and easily removed later for the examination of the seedlings. Frit fly injury, which may increase the difficulty of examination for leaf stripe, is avoided by sowing as early as possible, although in some seasons early sowing was almost impossible on account of the condition of the land. Even with these precautions there are such factors as aphid attack and injury by soil pests such as slugs, &c., which tend to reduce the exactitude of field trials below that of carefully controlled pot experiments. The method described below was used in 1936-7 and has been found to be the most satisfactory; that used for 1935 was somewhat different and is briefly referred to in the section dealing with the results.

Details of the method.

Preparation of plots. Each plot is 4 ft. (1.3 m.) wide and consists of a number of drills sown with the different seed samples under test, the drills being 16 in. (40 cm.) apart. The length of the plot will therefore depend upon the number of samples used. As the test is replicated five times, five plots in all are required. A distance of 2 yards is left between the plots so as to allow for comfortable working conditions. The plots are thoroughly raked before sowing in order to remove the larger stones and to prepare as suitable a seed bed as possible.

Sowing. Each drill is made with a hoe, care being taken in its preparation to ensure an even depth of sowing. The seed is sown from small paper envelopes, each envelope containing the necessary quantity of seed required for a 4-ft. length of drill. After sowing, a rake is used to close each drill and, finally, the whole plot is gently raked and well rolled.

Using a normal sample of Scottish Pure Line Potato Oats and a sowing rate of 16 st. per statute acre (250 kg. per hectare) it has been computed that in the neighbourhood of 2.75 million seeds are sown. If a spacing of 6 in. (15 cm.) is allowed between the drills, this is equivalent to the sowing of approximately 100 seeds or 3.4 gm. of seed to each yard length of drill. This sowing rate was selected for the field trials in order to approach as nearly as possible to conditions obtaining in practice. The seed is sown by weight and not by number in order to avoid the labour of counting—a 4-ft. length of drill sown with 4.5 gm. of seed being chosen so as to ensure that at least 100 seeds are sown in all cases.

Order of sowing. The test for each treatment is replicated five times, one drill in each plot being sown with similarly treated seed. In order to obtain a randomized order of sowing, all the envelopes for one plot are placed in a

bag and thoroughly mixed. The samples are then sown in the order in which they are drawn from the bag.

Netting the plots. Immediately after completion the plots are covered with wire-netting in order to prevent damage by birds and other pests. Netting of 1.0 in. (2.5 cm.) mesh and 72 in. (2 m.) wide is suitable for this purpose. It is first stretched flat over the plot, the ends and edges then being buried a few inches in the soil by the digging of shallow trenches along the ends and edges of each plot. The soil removed from the trenches is firmly replaced after the wire has been let in, thereby holding it in position. The netting is then raised to form a sort of domed shaped covering to the plots by supporting it on canes inserted in the soil of the plot at suitable intervals (see Pl. XXVIII, Fig. 2).

Care after sowing. In some seasons the plots may require an occasional weeding before counts are made.

Estimation of disease. When the primary phase of the disease has reached its maximum intensity each plant is pulled, examined for the disease (using the precautions outlined for the pot method), and classed as healthy or diseased. Tiller production must be carefully watched for in order to avoid the counting of tillers as individual plants. All the plants in each 4-ft. length of drill are examined in this way, the extent of the occurrence of the disease being afterwards calculated on a percentage basis.

COMPARATIVE RESULTS

1935

General.

Laboratory method. This method was not used in 1935 as its introduction into routine practice was not effected until the following year. In 1936, however, it was applied to a selected number of the seed samples used in 1935 which had been stored for future reference. The results are included in the table for 1935; they are satisfactory and do not appear to have been influenced by the over-year storage of the seed.

Pot-culture method. Tests by this method in both moist and dry soil were begun on the same date (March 21), 200 seeds (i.e. two pots) being used for each sample. Owing to the slower development of the seedlings in the dry soil this test required 53 days (i.e. 12 days longer than that in the moist soil).

Field method. In 1935 the field method adopted consisted of sowing a 12-yd. length of drill at the rate of 224 lb. per acre with each of the seed samples tested, the drills being spaced at 18-in. intervals for convenience of working. Three lengths, each of 1 ft., were selected at random from each drill for the purpose of recording results.

Fungicides under test. Apart from the range of proprietary materials which are recorded under code numbers, formalin, cuprous oxide, and talc were included in the tests for this year. Commercial formalin (40 per cent. formaldehyde) was supplied at the strength of 1 part in 320 parts of water

TABLE I

Tests with Seed Disinfectants for the Control of Helminthosporium of Oats

The quantities of dry material refer to the weight per 42 lb. of grain
(2 oz. per 42 lb. = 3.0 gm. per kg.)

Results for 1935

Material tested.	Method and rate of application.	Percentage of Helminthosporium disease.			
		Laboratory method.	Pot-culture method.		Field method.
Control	—	42.8	44.0	48.0	30.0
Talc	Dry, 1 oz.	—	34.0	41.5	25.0
"	Dry, 2 "	—	42.0	42.0	34.9
"	Dry, 4 "	—	38.5	34.5	17.5
Cuprous oxide	Dry, 3 "	10.8	27.0	42.5	14.7
Formalin	Wet, sprinkle	9.2	12.0	15.0	12.0
A1	Dry, 2 oz.	—	0.0	9.0	2.8
A2	Dry, 2 "	1.0	0.0	0.0	0.8
A3	Dry, 2 "	—	0.0	0.5	0.9
A4	Dry, 2 "	—	0.0	4.5	4.4
A5	Dry, 1 "	38.4	36.5	46.5	20.7
A6	Dry, 2 "	41.6	30.5	44.0	23.0
A7	Wet, short wet	—	0.0	0.0	1.7
A7	Wet, sprinkle	—	0.0	0.5	3.3
A7	Wet, steep	—	0.0	0.0	6.9
B1	Dry, 2 oz.	—	0.0	10.0	3.3
C1	Dry, 2 "	—	1.5	11.0	0.0
D1	Dry, 2 "	—	0.0	0.0	3.8
D2	Dry, 1 "	—	0.0	0.0	0.0
D3	Dry, 0.5 oz.	—	0.0	0.0	2.7
D4	Dry, 2 oz.	—	2.5	10.0	3.5
D5	Dry, 2 "	—	0.0	0.0	2.3

by the sprinkle method, while the talc used was supplied as a pure chemical in a very finely divided form. It was intended to use cuprous oxide as specified by Horsfall *et alia* (1934), but as this material could not be obtained the nearest obtainable from British Drug Houses was used.

Miscellaneous data.

Seed sample. Scottish Pure Line Potato Oats (Agnew II, 1933 crop).

Pot-culture method—moist soil. Date of sowing, March 21; date of cutting, May 1; duration of test, 41 days. Soil moisture content, 47.7 per cent. saturation. Mean temperature, 9.3° C.

Pot-culture method—dry soil. Date of sowing, March 21; date of cutting, May 13; duration of test, 53 days. Soil moisture content, 26.7 per cent. saturation. Mean temperature, 10.1° C.

Field method. Date of sowing, April 12; date of cutting, May 24; duration of test, 42 days.

The taking of the results from the field trial in 1935 was left until rather

late owing to unsettled weather. It is believed that by the time the counts were made a certain amount of secondary infection had taken place, thus accounting for the slightly irregular nature of the results for this year. This difficulty did not arise in the two subsequent years.

1936

General.

Laboratory method. Tests using this method were carried out according to the recommendations.

Pot method. These tests were also carried out according to schedule except that the dry soil-high temperature sowing was made on March 31. This was

TABLE II

Tests with Seed Disinfectants for the Control of Helminthosporium of Oats

Quantities as in Table I

Results for 1936

Material tested.	Method and rate of application.	Percentage of Helminthosporium disease.			
		Laboratory method.	Pot-culture method.		Field method.
			Moist soil low temp.	Dry soil higher temp.	
Control	—	43·0	21·0	42·0	5·0
Talc	Dry, 2 oz.	53·8	22·8	43·0	7·6
MHg 367 (Silica)	Dry, 2 „	9·2	11·6	39·6	3·6
Formalin	Wet, sprinkle	20·6	6·0	26·2	3·2
A1	Dry, 2 oz.	0·0	0·0	0·6	0·8
MHg 365 (A1)	Dry, 2 „	0·0	0·0	0·0	0·0
MHg 364 (A1 modified)	Dry, 2 „	0·0	0·0	0·2	0·0
A2	Dry, 2 „	0·0	0·0	0·0	0·0
MHg 362 (A2)	Dry, 2 „	0·2	0·0	0·0	0·4
A7	Wet, short wet	0·2	0·0	0·0	0·2
A7	Wet, sprinkle	5·0	0·2	0·8	0·6
A7	Wet, steep	0·2	0·0	0·0	0·2
C1	Dry, 2 oz.	0·0	0·2	0·8	0·0
MHg 366 (C1)	Dry, 2 „	0·2	0·0	2·0	0·8
D5	Dry, 2 „	0·0	0·0	0·2	0·0
MHg 361 (D5)	Dry, 2 „	0·0	0·2	0·0	0·2
MHg 363	Dry, 2 „	0·0	0·0	0·4	0·4

rather too early, and owing to the low spring temperatures which prevailed in 1936, a very low mean temperature was recorded for the test.

Field method. The field tests were laid down in accordance with the recommendations.

Fungicides under test. The only important change made with regard to the fungicides tested in 1936 was the inclusion of a number of materials submitted

by Dr. Martin of the Research Station, Long Ashton. On arrival these products proved to be, in the main, identical with those which had been under observation in Northern Ireland previously and which had been submitted to testing in 1935. In the table of results Martin's samples are listed under numbers prefixed by MHg. Where these materials were identifiable as proprietary products, the code number of the product is given in brackets.

Miscellaneous data.

Seed sample. Scottish Pure Line Potato Oats (Agnew II, 1933 crop).

Pot method (moist soil—low temperature). Date of sowing, March 11; date of cutting, April 30; duration of test, 50 days. Soil moisture content, 52.5 per cent. saturation. Mean temperature, 8.0° C.

Pot method (dry soil—high temperature). Date of sowing, March 31; date of cutting, May 12; duration of test, 42 days. Soil moisture content, 32.8 per cent. saturation. Mean temperature, 9.7° C.

Field method. Date of sowing, May 6; date of cutting, June 6; duration of test, 31 days.

1937

General.

Laboratory method. Tests using this method were carried out according to the recommendations.

Pot method. These tests were also carried out according to schedule. The results from the second pot test were not very satisfactory. The very dry soil recommended (25 per cent. saturation) is rather on the dry side for the regular growth of the seedlings and it was decided to work this year with soil a little moister. It was made too moist (45 per cent. saturation), with the result that the disease was not so severe as with drier soil.

Field method. The field tests were laid down in accordance with the recommendations.

Fungicides under test. Except for the increase in the number of proprietary materials under trial the only departure made for this year was the testing of a sample of cuprous oxide answering to the specification laid down by Horsfall (1934).

Miscellaneous data.

Seed sample—Scottish Pure Line Potato Oats (Biglees 1936 Crop).

Pot method (moist soil—low temperature). Date of sowing, March 25; date of cutting, May 10; duration of test, 46 days. Soil moisture content, 53.3 per cent. saturation. Mean temperature, 8.6° C.

Pot method (drier soil—high temperature). Date of sowing, June 9; date of cutting, July 10; duration of test, 31 days. Soil moisture content, 43.0 per cent. saturation. Mean temperature, 13.8° C.

Field method. Date of sowing, May 13; date of cutting, June 17; duration of test, 35 days.

TABLE III

Tests with Seed Disinfectants for the Control of Helminthosporium of Oats

Quantities as in Table I

Results for 1937

Percentage of Helminthosporium disease.

Material tested.	Method and rate of application	Pot-culture method			
		Laboratory method.	Moist soil, low temp.	Dry soil, high temp.	Field method.
Control	—	20.0	19.8	14.4	23.8
Talc	Dry, 2 oz.	16.0	27.4	14.0	20.7
Cuprous oxide	Dry, 2 „	5.0	25.0	—	16.1
Formalin	Wet, sprinkle	19.0	12.8	11.6	13.0
A2	Dry, 2 oz.	0.0	0.0	0.0	0.3
A7	Wet, short wet	0.0	0.0	0.2	0.3
A7	Wet, sprinkle	12.0	0.6	0.6	0.3
A7	Wet, steep	0.0	0.0	0.0	0.1
A8	Dry, 2 oz.	0.0	0.0	0.2	0.1
A9	Dry, 2 „	0.0	0.0	0.0	0.2
A10	Dry, 2 „	0.0	0.0	0.0	0.2
A11	Dry, 2 „	1.0	0.0	0.0	0.1
A12	Dry, 2 „	0.0	0.2	0.0	0.5
A13	Dry, 2 „	0.0	0.0	0.0	0.0
B2	Dry, 2 „	0.0	0.0	0.4	0.5
B2	Wet, short wet	—	—	0.8	—
C1	Dry, 2 oz.	0.0	1.2	1.6	2.3
C2	Dry, 2 „	—	—	0.2	—
D5	Dry, 2 „	0.0	0.0	0.0	0.0
E1	Dry, 1 „	0.0	0.4	0.2	0.0
E2	Dry, 2 „	0.0	0.0	0.0	0.3
E3	Dry, 1 „	0.0	0.2	0.0	0.5
E4	Dry, 1 „	17.0	21.6	—	20.0
E5	Dry, 2 „	33.0	19.6	—	19.4

The results given in Tables I-III for the pot and field methods are the calculated *means* from the total number of replications of pots or plots used for each sample. In order to show the nature of the detailed results obtained in the course of the work they are presented below in full for the pot test (moist soil—low temperature) 1936 and the field trials for 1937.

SCHEDULE OF TESTING

Choice of seed sample. A sample of seed oats heavily infected with *H. Avenae* is the first essential, and the laboratory method has been found to be extremely useful in the selection of a suitable sample. It is suggested that the sample should contain at least 15 per cent. of infected grains as determined by the laboratory method during the winter season following the harvesting of the crop. If stored under dry conditions the sample will serve for at least two seasons, and if at the end of this period the germination remains unimpaired it may be used for a third season. *H. Avenae* does not appear to die in seed stored for long periods.

TABLE IV

Detailed Results of the Pot-culture Test (Moist Soil—Low Temperature), 1936, expressed as Percentages of Helminthosporium Disease

Material tested.	Pot 1.	Pot 2.	Pot 3.	Pot 4.	Pot 5.
Control	20	19	21	21	24
Talc	20	23	19	29	23
MHg 367 (silica)	9	11	12	15	11
Formalin	3	6	12	3	6
A1	0	0	0	0	0
MHg 361 (A1)	0	0	0	0	0
MHg 364 (A1 modified)	0	0	0	0	0
A2	0	0	0	0	0
MHg 362 (A2)	0	0	0	0	0
A7 (short wet)	0	0	0	0	0
A7 (sprinkle)	1	0	0	0	0
A7 (steep)	0	0	0	0	0
C1	0	1	0	0	0
MHg 366 (C1)	0	0	0	0	0
D5	0	0	0	0	0
MHg 361 (D5)	0	0	0	0	1
MHg 363	0	0	0	0	0

TABLE V

Detailed Results of Field Trial, 1937, expressed as Percentages of Helminthosporium Disease

Material tested.	Plot 1.	Plot 2.	Plot 3.	Plot 4.	Plot 5.
Control	18.8	28.7	31.4	25.0	14.9
Talc	19.7	18.3	25.0	19.0	21.6
Cuprous oxide	15.7	19.0	16.8	17.4	11.7
Formalin	13.0	12.1	9.9	14.0	16.1
A2	0.0	0.0	0.7	0.0	0.8
A7 (short wet)	0.0	0.0	0.0	0.0	1.0
A7 (sprinkle)	0.8	0.0	0.0	0.8	0.0
A7 (steep)	0.0	0.8	0.0	0.0	0.0
A8	0.0	0.0	0.7	0.0	0.0
A9	0.8	0.0	0.0	0.0	0.0
A10	0.0	0.0	0.0	0.0	0.8
A11	0.0	0.0	0.7	0.0	0.0
A12	0.0	0.0	0.0	2.5	0.0
A13	0.0	0.0	0.0	0.0	0.0
B2	0.0	0.0	0.7	1.6	0.0
C1	2.4	1.5	0.7	4.4	2.4
D5	0.0	0.0	0.0	0.0	0.0
E1	0.0	0.0	0.0	0.0	0.0
E2	0.0	0.0	1.5	0.0	0.0
E3	0.0	0.0	0.8	0.0	1.7
E4	22.3	24.0	12.4	19.5	21.6
E5	18.0	21.0	20.3	16.6	21.0

Seed treatment. Seed treated by the dusting method is shaken with the requisite quantity of the fungicide for three minutes in a conical flask loosely plugged with cotton-wool; wet treatments are applied by methods appropriate for use with small quantities of seed. A suitable quantity of grain for each

test is 250 gm.; this is ample for the laboratory, pot, and field tests, and leaves a sufficiency for any other test which may be desirable as well as a surplus for subsequent storage. Treated at the rate of 2 oz. per bushel (42 lb.), 250 gm. of seed oats require 0.75 gm. of a dust fungicide.

The tests.

The laboratory test. This can be made at any time and is normally carried out soon after the seed has been treated. For this test 100 grains are used. If the results show a complete control of *H. Avenae* the sample is ear-marked for pot and field tests. If 5 per cent. or more of the grains are affected with viable *H. Avenae*, it is probable that the fungicide is of little value, and the sample is rejected without further testing. Should the percentage fall between 0 and 5, then a re-test is made and the sample rejected after this second test, unless the amount of infection recorded is 1 per cent. or less, in which case it is ear-marked for pot and field tests. It is not suggested that these recommendations should be rigidly observed in every case. They should be regarded as a guide to the interpretation of the results, and although cases may arise which require special consideration their adoption in general has proved useful and satisfactory.

Pot and field tests. Those materials which satisfy the conditions of the laboratory test are automatically subjected to pot and field tests. For each of these tests, carried out by the methods already outlined in detail, 500 grains are used. The interpretation of the results obtained from these tests is discussed below.

DISCUSSION

In this paper a comprehensive biological technique is presented whereby the value of a seed dressing for controlling *Helminthosporium* disease of oats may be accurately assessed within the space of one growing season. By use of the laboratory method, which occupies ten days and may be carried out at any time, materials of little or no value may be selected and rejected at once without recourse to further trial. Promising materials are submitted to three further tests, two of which are pot trials and the third a field test. Should the conditions of the field test be such as to produce insignificant attack in the controls, there are the additional results obtained by the pot-culture method, where an epidemic outbreak is ensured. Over the experimental period of three years a high degree of correlation has been found to exist between the results obtained by all three methods.

Of the fungicides tested, the only satisfactory ones were among proprietary materials containing organic mercury compounds. The effectiveness of some of these materials was as nearly perfect as can be expected in practice. Formalin, which is such a satisfactory fungicide for smut of oat (Muskett and Cairns, 1932), did not give satisfactory control of *Helminthosporium*. Compounds used as fillers, such as talc and silica, appear to have little fungicidal value, although the sample of silica supplied by Martin in 1936 did appear

to exercise some measure of control. Cuprous oxide, as supplied by both British Drug Houses and Dr. Horsfall (Messrs. Röhm and Haas of Philadelphia, U.S.A.), failed as a fungicide and also caused a noticeable growth depression in the seedling crop. Copper fungicides would appear to be unsuitable for general use with the oat crop; damage following the use of copper sulphate solution as a sprinkle, and copper carbonate dust, was recorded by Muskett and Cairns in 1932.

For the control of *Helminthosporium* disease it is tentatively suggested that a satisfactory fungicide should not allow an average of more than 0.2 per cent. of the disease to escape control when submitted to the pot trials and the field test. Such a requirement may seem severe, although it will be seen from the results that more than one proprietary material satisfied these conditions.

The success of a seed dressing, however, may not depend entirely upon its usefulness in controlling one specific seed-borne disease; there are other diseases and other factors which may have to be taken into account, the joint consideration of which may necessitate some modification of the requirements demanded by each disease or factor when considered in isolation. Large industrial undertakings occupied in the manufacture of these materials appear to be aiming at the production of universal seed dressings capable of the successful treatment of as many seed-borne diseases as possible. In some measure this object has been attained in the organic mercury dressings of most recent introduction, the present tendency being to keep the mercury content of the preparation as low as is compatible with fungicidal efficiency. An important step forward will have been made when a universal seed disinfectant, non-poisonous to man and animals, is forthcoming.

It is therefore evident that the evaluation of seed dressings for the control of *Helminthosporium* disease of oats is but one aspect of a much wider problem. For cereal crops alone an improved and more precise technique is desirable for such diseases as bunt in wheat, covered smut in barley, loose and covered smuts in oats, and other *Helminthosporium* diseases, &c. Even when the requirements demanded by the better known seed-borne diseases of cereals have been met, there still remain aspects of the general problem upon which further information is desirable, for instance those seed-borne diseases about which little is yet known and which still require elucidation. That such diseases exist and are of some importance appears to be fully justified by the number of cases where the disinfection of seed prior to sowing produces a distinctly beneficial effect upon the resultant crop not due apparently to the control of better-known troubles. Cases of this type have been met with in the oat crop from time to time by the writer. In the spring of 1937 a number of oat seed samples under the conditions of pot test produced a much better crop of seedlings as the result of disinfection. *H. Avenae* was present to some extent in all these samples, but in most cases the infection was light and it was found difficult to account for the very noticeable improvement as being due entirely to the control of this disease. The infection of

seeds by diseases of this type is largely dependent upon the season, while harvesting conditions probably influence their prevalence in some measure, thus accounting for the very significant differences occurring in the intensity of the infection from year to year.

Another interesting experience of 1937 suggests that seed disinfection may serve to protect a cereal crop from pest damage without exercising any direct control of the actual pest involved. About nine-tenths of a large field was sown with disinfected seed oats, while the remaining tenth was sown with seed from the same sample without disinfection. The crop from the control seed was a complete failure, while almost a full crop was produced by the disinfected seed. A very marked superiority of the seedlings from the disinfected seed was noticed early in the season, and as leatherjacket grubs (*Tipula* spp.) were found in the soil it was suggested that the disinfection of the seed had exercised control over the damage caused by the grub. On examining the field, leatherjackets were found to be present in very large numbers, but they were as numerous in that part of the field sown with disinfected seed as in the portion where the seed had not been disinfected. The explanation of this case appears to lie in the fact that the braird produced by the disinfected seed was thick, strong, and vigorous, and far more able to withstand the grub attack than the thinner, weaker, and less healthy braird produced by the non-disinfected seed.

The possibility of seed disinfection causing subsequent injury to the crop must not be overlooked, and makes a case for the growing of the crop to maturity for the determination of yield. As field trials for the estimation of infection by smut diseases necessitate the full growth of the crop, a somewhat similar technique should prove satisfactory for the conduct of yield trials. The determination of primary infection by *H. Avenae* is somewhat exceptional in requiring only the growth of the crop as far as the seedling stage.

SUMMARY

1. Three methods have been evaluated for the control by seed disinfectants of the primary phase of *Helminthosporium* disease of oats. By the Laboratory Method, which may be carried out at any time during the year, ineffective fungicides can be assessed within an experimental period of ten days and rejected without further testing. The Pot-culture Method ensures an epidemic outbreak of the disease in the control plants so that the fungicide can be submitted to a rigorous test. Two pot tests are made under conditions of moist soil and early in the year, i.e. at low temperature, and the other with drier soil and during the late spring or early summer, i.e. at high temperature conditions. The conditions of soil moisture and temperature employed in the first test encourage the development of the disease; the results of the second test provide a check on those from the first. A detailed account of the methods used is given.

2. Over the experimental period of three years a high degree of correlation has been obtained between the results of the three methods.
3. By the scheme put forward a fungicide may be thoroughly tested in one growing season.
4. Certain proprietary fungicides containing mercury in an organic combination gave almost perfect control of the primary phase of the disease, while formalin, cuprous oxide, talc, and silica have been shown to be unsatisfactory.
5. A standard performance is suggested for a fungicide satisfactory in its control of *Helminthosporium* disease. The value of this standard is considered in relation to the control of seed-borne diseases in general.

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Thanks are due to Dr. Martin and Dr. Horsfall for supplying fungicides for test purposes. Messrs. Bayer Products, Ltd., F. W. Berk & Co., Ltd., Imperial Chemical Industries, Ltd., Lunevale Products, Ltd., and Schering, Ltd., supplied the various proprietary materials used in the course of the work.

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EXPLANATION OF PLATE XXVIII

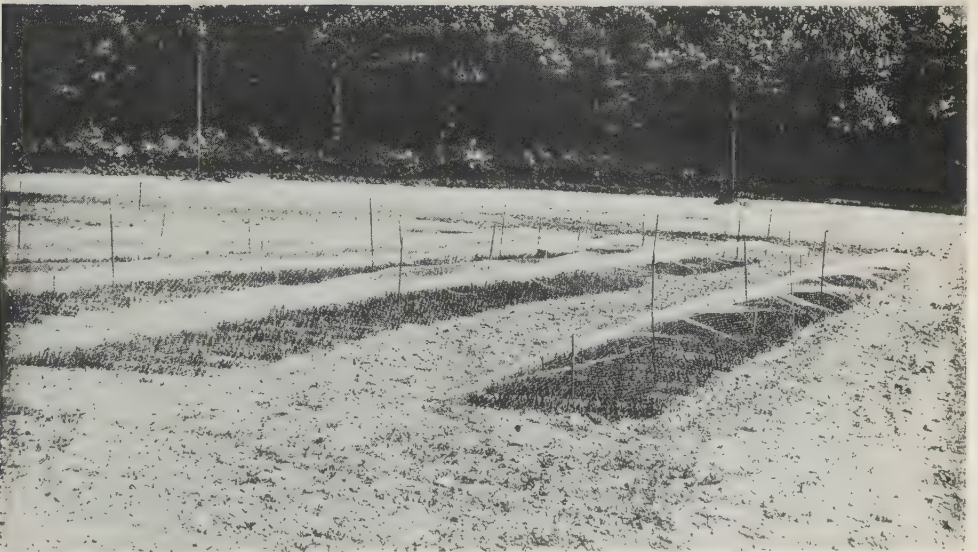
Illustrating Dr. A. E. Musket's paper on 'Biological Technique for the Evaluation of Fungicides. I. The Evaluation of Seed Disinfectants for the Control of *Helminthosporium* Disease of Oats.'

Fig. 1. The Pot-culture Test in progress (1936).

Fig. 2. Field Plots (1937) at the Agricultural Research Institute, Hillsborough.



1



2

Huth, Stubbs X, Kent

MUSKETT — EVALUATION OF FUNGICIDES.

The Measurement of Assimilation and Translocation in Tomato Seedlings under the Conditions of Glass- house Culture

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With three Figures in the Text

INTRODUCTION

THE methods described by Bolas and Melville (1933) for the measurement of assimilation rate, using a 'paired-plant', dry weight method, have been applied to the measurement of assimilation rate in seedling tomato plants over the period 1932-4. While it has now become apparent that a modified technique would yield more precise results, it seems desirable to set forth these preliminary results and to indicate briefly the extent to which this type of experiment is likely to throw light on the physiological effects of the conditions obtaining in normal glasshouse practice.

EXPERIMENTAL DETAILS

The procedure was the same as that already described. Seeds of the tomato, variety E.S. 1, were sown in seed-boxes and the seedlings transferred to 3½-inch pots when the first two foliage leaves had appeared. The soil used was a compost containing baked soil from the cucumber houses, to which had been added a little bonemeal. No attempt at environmental control was made, apart from the routine practices of the nursery staff, which included heating of the house in the colder months, and the shading and cooling induced by spraying the glass with flour wash in the summer months. Care was observed in maintaining an adequate water-supply to the plants.

It was necessary to select an arbitrary stage in the plants' development at which the assimilation experiment was to be made. For convenience, plants having 7-8 expanded leaves were always used. It is recognized that plants at this morphological stage taken at different times of the year must be of very different ages. Thus plants sampled in August might be 4 weeks old,

whereas plants of the same morphological stage taken in December would be 7–8 weeks old. Nevertheless, Richards (1938) has pointed out that plants at the same stage of development are more nearly at the same stage of physiological ontogeny than plants of the same age. If this is true for plants whose development is controlled by different levels of mineral supply it may be expected to be true of plants whose development has been controlled primarily by the interaction of light, temperature, and length of day.

On the other hand, the permanent effect of the previous external history must undoubtedly introduce a source of error in these experiments. Plants taken at different times have been found to show differences in dry weight, fresh weight, percentage water content, leaf-area, and in the ratios of weight of stem, leaf, and root. It thus appears highly probable that plants with these differences will not react in an exactly similar manner to any given combination of two factors such as light and temperature. The observed assimilation rates must therefore be taken as recording the response of plants grown under a wide range of seasonal conditions.

Samples of not less than 20 carefully paired plants were taken from a population of 150–250 individuals. At 9.0 a.m. on the day of the experiment, one member of each pair was taken at random from the group and the fresh and dry weights of stem, of leaf, and of root were determined. At 30- or 60-minute intervals the light intensity in the greenhouse was recorded, using a Holophane lumeter, and the mean temperature was computed from a thermograph record. The remainder of the plants were sampled at 4.0 p.m.

In Table I estimates of the assimilation and translocation rates are recorded for thirty-two experiments performed at all seasons over a period of two years, together with the mean light intensity and mean temperature during the seven-hour assimilation period.

DISCUSSION OF RESULTS

Assimilation rate.

The assimilation rate has been expressed as

$$\frac{\text{Gain in dry weight of whole plant} \times 100}{\text{Morning dry weight of leaf system.}}$$

This fraction seems to be a more satisfactory estimate of assimilation rate than simply the percentage increase in dry weight of the whole plant, since account is taken of the bulk of the assimilatory tissue. Leaf-area measurements were only made in seven of these experiments, and for comparative purposes the conventional measure of assimilation rate has been calculated from the data of these experiments and recorded in Table II. The leaf-area measurements were made using the phyllometer method of Bolas and Melville (1933) and the number of milligrams of carbon dioxide assimilated per square decimetre of leaf surface per hour was calculated on the assumption that the whole of the dry-matter increase in the seven-hour period was due to the

TABLE I

Date of experiment.	Mean temp. (° C.).	Mean light intensity (foot-candles).	Gain in dry wt. whole plant $\times 100$		Gain in dry wt. stem + root $\times 100$		Dry wt. stem + root $\times 100$	
			initial dry wt. leaf system		gain in dry wt. plant		dry wt. plant	
22/8/32	19.4	548	36.0		31.4		38.9	
31/8/32	22.8	779	21.2		22.2		24.6	
19/9/32	14.9	271	19.9		21.6		39.8	
21/9/32	19.0	812	18.6		22.0		39.8	
7/10/32	20.6	193	17.9		25.6		40.3	
13/10/32	21.1	428	27.2		28.6		37.5	
14/10/32	21.4	700	21.6		26.8		39.4	
24/10/32	19.0	172	14.0		25.0		37.1	
2/11/32	17.8	158	12.8		34.4		37.6	
14/11/32	16.4	45	1.1		36.4		36.4	
5/12/32	18.0	97	9.6		16.7		43.8	
19/1/33	17.0	57	19.1		61.3		46.1	
4/5/33	28.3	1184	44.4		27.7		39.8	
11/5/33	24.0	878	31.8		22.9		37.8	
25/5/33	22.4	1056	16.4		17.0		37.2	
9/6/33	24.5	233	29.1		31.2		38.5	
14/6/33	21.8	1049	19.6		25.0		37.8	
7/7/33	26.0	1441	36.4		30.6		40.0	
20/7/33	24.5	579	21.2		27.4		39.6	
27/9/33	22.5	146	22.9		27.0		40.7	
4/10/33	23.9	823	29.7		23.8		40.5	
11/10/33	23.4	516	43.7		30.9		41.2	
25/10/33	17.6	132	2.2		10.9		41.0	
10/11/33	17.7	191	7.7		44.2		41.6	
16/11/33	19.2	125	1.8		(172.0)		(44.5)	
29/11/33	18.1	82	13.3		52.5		47.4	
18/1/34	17.3	131	14.1		17.3		39.6	
28/2/34	18.2	126	12.5		43.2		39.3	
2/3/34	19.7	289	14.6		13.0		38.4	
12/4/34	23.7	665	38.4		24.0		36.8	
27/4/34	21.0	271	25.4		40.6		37.5	
16/5/34	26.8	808	28.8		21.2		37.8	
Mean	20.9	468	21.0		28.5		39.2	

TABLE II

Date of experiment.	Mean light intensity (foot-candles).	Mean temp. (° C.).	Mean leaf area (sq. cm.).	CO ₂ assimilated (mg. per sq. dm. per hr.).	Gain in dry wt. whole plant $\times 100$.	
					initial dry wt. leaf system	
22/8/32	548	19.4	327	9.6	36.0	
31/8/32	779	22.8	339	8.8	21.2	
19/9/32	271	14.9	311	8.2	19.9	
21/9/32	812	19.0	291	7.6	18.6	
7/10/32	193	20.6	342	7.8	17.9	
13/10/32	428	21.1	315	9.2	27.2	
14/10/32	700	21.4	326	7.2	21.6	
Mean	533	19.9	322	8.3	23.2	

production of a hexose sugar. The mean assimilation rate of seven experiments performed in the autumn of 1932 was 8.3 mg. CO₂/sq. dm./hr., which corresponds to an increase in dry weight of the whole plant of 23.2 per cent. expressed in terms of the initial leaf weight in seven hours. No account has been taken of dry-weight losses due to respiratory activity.

In Fig. 1 the assimilation rates have been plotted as ordinates against time

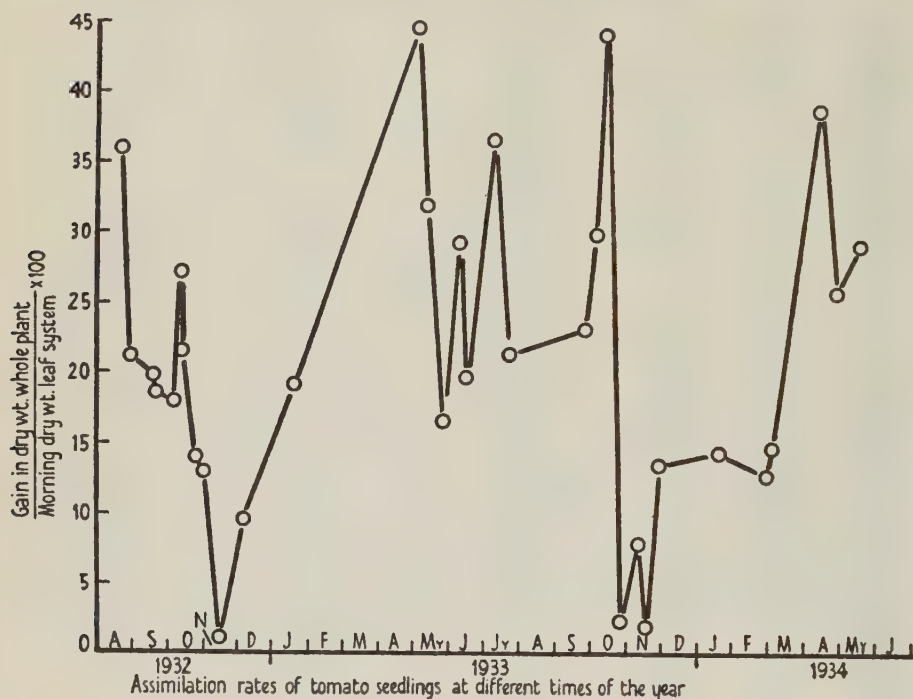


FIG. 1. Assimilation rates of tomato seedlings at different times of the year.

of year as abscissa. A marked seasonal trend in assimilation rate is evident, minimal values being recorded in November.

Bearing in mind the limitations imposed upon these data by the seasonal variations in plant-form and structure, it is of interest to analyse the mean response of the plants to light and temperature during a seven-hour assimilation period.

The effect of light and temperature on assimilation rate.

It was found that a linear regression could be used to express the effects of light and temperature on assimilation rate thus:

$$y = 0.005061x_1 + 2.1282x_2 - 25.765,$$

where

$$y = \frac{\text{gain in dry weight of whole plant} \times 100}{\text{morning dry weight of leaf system}}$$

$$x_1 = \text{mean light intensity in foot-candles}$$

$$x_2 = \text{mean temperature in } ^\circ \text{C.}$$

Analysis of Variance

	D.F.	S. of sq.	M. sq.	'z.'	0.1% pt.	1% pt.
Linear regression	2	2265.383	1132.692	1.4670	1.0903	0.8451
Deviations from regression . .	29	1747.586	60.2616			
Total	31	4012.969				

Whence the standard error of a single experiment = ± 7.76 .

Analysis of Multiple Regression. I

A. Regression of light on assimilation (temperature not eliminated)	1	1573.603	1573.603	1.4814	1.2936	1.0139
B. Partial regression with temperature (light effect eliminated)	1	691.780	691.780	1.2203	1.2936	1.0139
Deviations from regression . .	29	1747.586	60.2616			
Total	31	4012.969				

(Testing A v. B plus deviations as 30 degrees freedom.
 „ B v. deviations directly.)

The partial regression of temperature with assimilation rate is found to be significant.

Analysis of Multiple Regression. II

A. Regression of assimilation on temperature (light not eliminated)	1	2212.083	2212.083	1.8034	1.2936	1.0139
B. Partial regression of light with assimilation (temperature eliminated)	1	53.300	53.300			
Deviations	29	1747.586	60.2616			
Total	31	4012.969				

(Testing A v. B plus deviations as 30 degrees freedom.
 „ B v. deviations directly.)

Whence the partial regression of light with assimilation is seen to be Non-significant.

Partial correlation coefficient light with assimila-

tion = 0.173 (not significant).

Partial correlation coefficient temperature with

assimilation = 0.629 (significant).

The introduction of second order terms into the regression equation did not reduce the error variance appreciably, and since the light coefficient in the linear regression has been shown to be insignificant, one may express the relationship within the limits of the experiments as

$$y = 2.1282x_2 - 25.765,$$

or as an inclined plane with temperature and light as ordinate and abscissa respectively, and assimilation rate as vertical height. A relief map of this plane is given in Fig. 2, the full lines representing 10 per cent. assimilation

rate contours calculated from the regression equation. These contours have only been inserted to cover the approximate range of light and temperature encountered in these experiments, and since photosynthesis may be expected to proceed at least up to 35° C. and 4,000 foot-candles, the very limited range of conditions obtaining in the greenhouse at Cheshunt is strikingly apparent.

It is unfortunate that in the present work no observations were made at high temperatures and low light intensities and vice versa, for if such points were available it might be possible to demonstrate that the straight line contours shown in Fig. 2 actually form part of curves resembling rectangular hyperbolae, which are the contours to be expected on *a priori* physiological grounds. In Fig. 2 the broken lines have been inserted to indicate the general type of contours which might be expected.

The only other plant upon which a detailed study of the interaction of light and temperature in controlling assimilation rate has been made is Lemna, which is a heliophilous plant. By adjusting the scale of the light axis in the solid model figured by Ashby and Oxley (1935) it becomes clear that the results obtained in the present work are not necessarily incompatible with those found for Lemna, or with those reported by Matthaei (1904) with cherry laurel leaves. In the latter work no absolute scale of light intensity is given so that direct comparisons are not possible.

From the unexpected result of these experiments concerning the unimportance of light intensity, above certain low minimal values, in controlling assimilation rate, one may deduce that this variety of tomato (E.S. 1) has been selected for its ability to assimilate well under low light intensities. It was actually selected for its growing and fruiting capacities under glass, by the Research Station, Cheshunt, which is situated in the Lea Valley where fog and mist are very prevalent. This variety of tomato must be regarded as a shade plant, despite the sunny habitat of its wild prototype.

Over the range of light and temperature encountered in these experiments, namely 45–1,440 foot-candles and 15–28.5° C., light thus appears to have no effect on assimilation rate whilst temperature was found to have a very profound effect. This statement is not necessarily true for all combinations of these light intensities and temperatures, for there was a very high natural correlation between light and temperature in the greenhouse ($r = 0.73$). On the other hand, it is well known that it is useless to maintain a high temperature in the winter months, for by so doing an unhealthy, soft plant useless for economic purposes is produced. It might appear, therefore, that the length of the light period (i.e. day length) is of greater importance than the actual light intensity, provided a certain minimal light intensity of 100–200 foot-candles is maintained at ordinary glasshouse temperatures. This is in accord with the results reported by Bewley (1936) with cucumber seedlings grown in January. Plants receiving four hours' additional daily illumination at light intensities of 70 and 280 foot-candles all showed marked increases in growth over the controls.

From the present data there is no evidence of either light intensity or temperature being anywhere near the supraoptimal level for assimilation, but while it is realized that assimilation rate, balanced growth, and fruiting bear

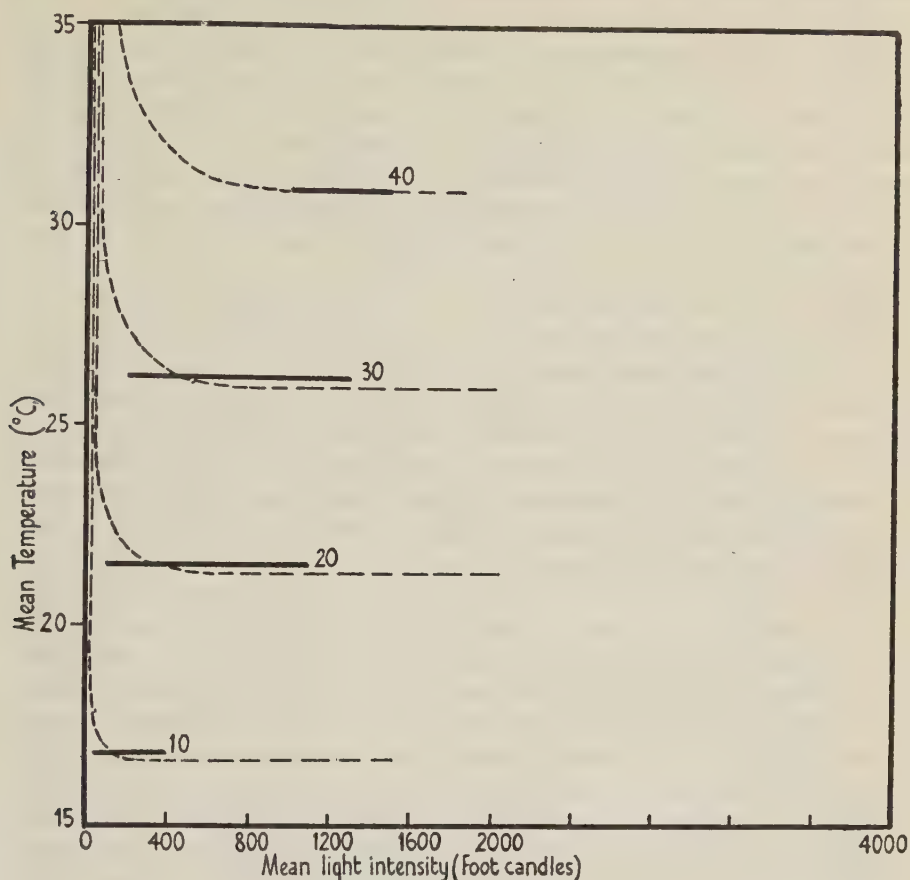


FIG. 2. Diagram of assimilation rate contours from light-temperature-assimilation-rate surface (for explanation see text).

no simple relation the one to the other, it may be suggested that a careful study of the effects of summer shading of the greenhouse might prove profitable.

The effect of other factors on assimilation rate.

In reviewing this work, it should be pointed out that the standard error of the mean percentage assimilation rate in any one of the experiments did not usually exceed ± 6 , hence the variance due to error among replicate plants, in the complete analysis, is unlikely to exceed 36. In other words, only slightly more than one-half of the variance recorded on p. 721 as due to deviations from the linear regression is accounted for by error

among the replicate plants. Since the fitting of second order terms to the regression did not appreciably reduce this error variance, it may be assumed that some other factors are exerting a measurable influence on assimilation rate. This is not unreasonable, since the only factors of which account was taken were light, temperature, soil composition, and the stage of development of the plant. Other factors which may influence the assimilation rate are: (1) atmospheric humidity; (2) soil moisture content, always adequate but never controlled in this work; (3) carbon dioxide content of the air; (4) the immediate past history of the plant, i.e. as determined by the conditions obtaining in the twenty-four hours preceding the assimilation period; (5) the general past history of the plant as determining type of plant, &c., as mentioned on p. 718.

Of these five the last two are probably of the greatest importance in the conditions of these experiments.

As has been mentioned earlier, in the assimilation rates measured in these experiments no account of the respiration losses has been made. It must not be presumed that such losses are likely to be negligible, for respiration rates exceeding 0.8 per cent. of the total dry weight per hour at 25° C. have been recorded for normal barley leaves by Gregory and Sen (1937), so that when the respiratory losses of the non-assimilating plant organs are also introduced, respiration may become an important source of error in experiments of this type.

Under certain conditions the magnitude of the previous day's assimilation and the length of the dark period immediately preceding the assimilation period may exert an important influence upon both respiration rate and the rate of photosynthesis during the time of the experiment. Matthaei (1904), referring to the work of Borodin, states that: 'Prolonged darkness produces a steady diminution in the amount of carbon dioxide given off by a leaf; but if such a starved leaf be allowed to assimilate actively even for an hour or so the respiration will at once increase greatly, although much additional reserve material may not have been produced. Thus the respiration of a leaf does not depend merely on the temperature, but is greatly influenced by recent assimilation or starvation.' Later work has served to emphasize the importance of these factors.

It may be suggested that deviations from the assimilation values in the winter months, predicted from the regression equation, are related primarily to the physiological disturbances accompanying a subnormal carbohydrate level. The latter will be induced by the tendency for a low assimilation rate to occur on the day preceding the experiment, and this will be accentuated by respiratory losses during the long nights. On the other hand, in the summer months one of the controlling factors is likely to be the degree of accumulation of assimilate on the preceding day, which again may tend to be accentuated by the shortness of the summer night, which may not be of sufficient duration to permit of complete utilization of all the available carbo-

hydrates in growth. Unfortunately neither light intensity nor total radiation are recorded at Cheshunt, so that it has not been possible to attempt correlations between assimilation rate and the conditions obtaining on the previous day; greenhouse temperatures on the day preceding are not available.

One measure of the past history factors is given by the morning water content of the tissues, and under certain conditions this may be correlated with assimilation rate, as Melville (1937) has shown. It seems probable, however, that the magnitude of this value, with plants receiving a normal length of night, will be largely conditioned by the integration of the whole of the plants' previous history, rather than by the history of the past twenty-four hours. No simple correlation could be demonstrated between the deviations of the observed from the predicted assimilation values and the morning water content of the tissues.

Estimations of the starch and sugar contents of the tissues prior to the assimilation period might prove valuable in assessing the importance of the carbohydrate level as a factor in photosynthesis.

The movement of assimilate into the stem and root.

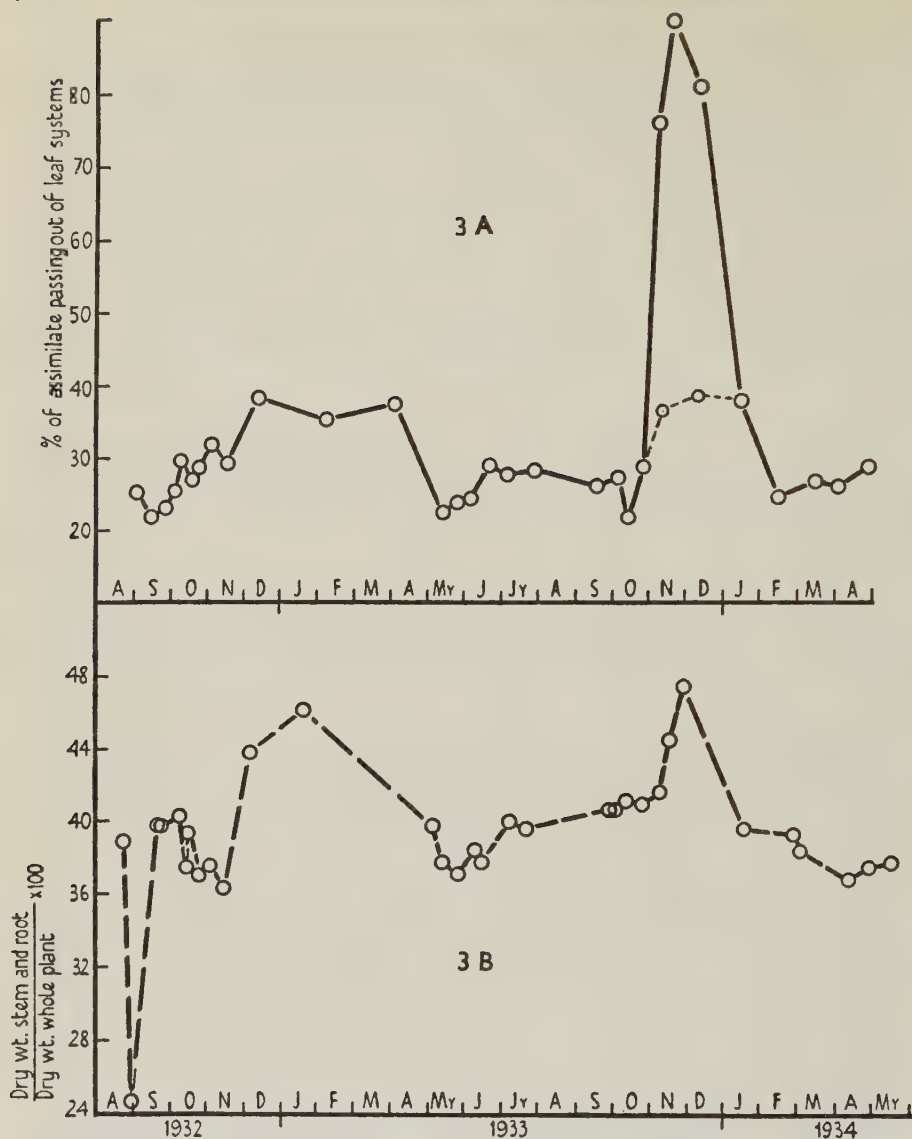
The data available enable one to calculate not the full translocation but that part of the translocated material which passes out of the leaf system to the root and stem. There must be very considerable translocation from one part of the leaf system to another, i.e. from the older to the younger rapidly developing leaves.

The assimilation carried on by the green stem being negligible, the fraction of the total assimilate passing into the stem and root or $\text{Gain in Dry Weight of Stem} + \text{Root} \times 100 / \text{Gain in Dry Weight Whole Plant}$ will be referred to as the percentage of assimilate translocated.

Data of the percentage of assimilate translocated. In Table I, col. 5, the numerical values of the percentage of assimilate translocated in the assimilation experiments are presented, and in Fig. 3A the 'running means' of these values (taken in threes) have been plotted graphically against the date of experiment.

In Fig. 3B the values of the fraction $\text{Dry Weight Stem} + \text{Root} \times 100 / \text{Dry Weight Plant}$ have been plotted against the date of experiment and are shown by a broken line. These values are presented numerically in Table I, col. 6.

It will be seen from Figs. 3A and 3B that there is a general correlation between the percentage of assimilate translocated in the seven-hour period and the proportion of the plant which is formed by stem and root. It is to be expected that the ratio of stem+root/plant will be determined by the integration of all the fractions of assimilate translocated throughout the previous history of the plant, and a measure of this integration has been obtained by taking 'running means' of the experimental values as stated above. By this method the large fluctuations manifest in the individual estimates of translocation rate have in Fig. 3A been considerably reduced. The greater height of the maximum.



FIGS. 3A and 3B. Fig. 3A. The percentage of assimilate passing out from the leaf system into the root and stem is plotted against season. Fig. 3B. The ratio of the weight of the stem and root to that of the whole plant is plotted against season.

of the translocation curve of Fig. 3A as compared with that of the dry weight distribution curve of Fig. 3B is due entirely to the inclusion of the experiment of November 16th, 1933, in which the percentage of assimilate translocated is recorded as 172. This figure is undoubtedly erroneous, since the maximum value possible is 100. With this point omitted, the curve takes the position indicated by the dotted line in Fig. 3B.

The similarity of the general trend of the two curves shown in Figs. 3A and 3B might suggest that the bulk of the assimilate that is moved out into the stem and root actually passes out of the leaf system during the period of assimilation. The mean values given in Table I, cols. 5 and 6, serve to emphasize this suggestion very strongly. Omitting the experiment of November 16, 1933, it will be seen that the mean of thirty-one experiments for the fraction of the assimilate translocated in the seven-hour assimilation period is 28.5 per cent. If the form of the plant is maintained approximately constant over a twenty-four hour period, then 39.2 per cent. of the assimilate may be taken as moving into the stem and root in twenty-four hours, for this is the fraction which the stem and root initially form of the whole plant. This leads to the deduction that of the total translocation of assimilate to be expected as a result of a seven-hour period of photosynthesis, 72 per cent. of that translocation occurs during the period of assimilation.

It has not been possible to demonstrate any simple correlation between light and temperature and the percentage of assimilate translocated. As has been pointed out above, this translocation fraction is subject to wide fluctuations, so that the result must not be taken as affording positive evidence against the existence of some relatively simple correlation.

Again, there is considerable evidence from many sources that the concentration gradient of carbohydrates from leaf to stem and root is a factor of prime importance in determining the rate of translocation; thus one might reasonably seek a correlation between the assimilation rate and the percentage of assimilate translocated. The limitations of this type of experiment are here very evident, for assimilation rate and the percentage of assimilate translocated, as defined above, are in fact only approximations and a correlation between the two is merely one between the absolute gain in dry weight of stem and root and the reciprocal of the initial leaf weight.

SUMMARY

1. The results of thirty-two experiments are recorded, in which the increase in dry weight of seedling tomato plants over a seven-hour period of natural light in a greenhouse has been determined using a 'paired plant' method.

2. A marked seasonal trend in assimilation rate is demonstrated, with minimal values in November.

3. A statistical analysis of the data leads to the conclusion that under the conditions of glasshouse culture and over the range of light and temperature encountered in these experiments, namely 45-1,440 foot-candles and 15°-28.5° C., light has no effect on assimilation rate, whereas temperature is positively correlated with assimilation rate. It is pointed out that this statement is not necessarily true for all combinations of these light intensities and temperatures, since there was a high correlation between light intensity and temperature in the glasshouse ($r = 0.73$, $p < 0.01$).

4. The effect of temperature on assimilation rate can be expressed by the linear regression equation,

$$y = 2.128 x_2 - 25.765$$

where $y = \frac{\text{Gain in dry weight whole plant in 7 hrs.}}{\text{Initial dry weight of the leaf system}} \times 100$

and $x_2 =$ mean glasshouse temperature in $^{\circ}\text{C}$.

The partial correlation coefficient of temperature with assimilation rate (with the effect of light eliminated) = 0.629.

5. The movement of assimilate into the stem and root is discussed and seasonal variations were demonstrated in the fraction $\frac{\text{Gain in Dry Weight Stem} + \text{Root} \times 100}{\text{Whole Plant}}$ which is taken as a measure of translocation. This measure in the seven-hour assimilation period tended to reach a maximum in the winter months. Seasonal variations in this ratio were found to run closely parallel with variations in the ratio of the dry weight of stem plus root to the dry weight of the whole plant.

6. The mean value (thirty-one determinations) of the fraction of assimilate passing from the leaf system into the stem and root in seven hours was 28.5 per cent. In these plants the dry weight of stem plus root was 39.2 per cent. of the dry weight of the whole plant.

In conclusion the authors desire to express their appreciation of the advice and interest accorded to them throughout the course of these investigations by Professor V. H. Blackman, Dr. W. F. Bewley, and Professor F. G. Gregory. Sincere thanks are also due to Mr. F. J. Richards for advice concerning the statistical treatment of the results and for a critical examination of the manuscript.

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Studies on the Nitrogen Metabolism of Plants

II. Interrelations among Soluble Nitrogen Compounds, Water and Respiration Rate

BY

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AND

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With fourteen Figures in the Text

INTRODUCTION

IN paper I (Petrie and Wood, 1938) of this series were presented the results of three experiments on nitrogen metabolism in the leaves of the grasses *Phalaris tuberosa* L. and *Lolium multiflorum* Lam. The aim of the experiments was to obtain quantitative information concerning the relations among the amounts of various nitrogen compounds present. The results were discussed in so far as they concerned the relation between the amount of protein in the leaves and certain other quantities that varied as the result of the experimental treatment; it was concluded that the protein content of the leaves increases with both amino-acid content and water content, and that, although neither a constant, nor perhaps even a drifting, steady state was attained in these experiments, such a state would probably be characterized by the same relationship.

There remains to be considered in this second paper the results for the soluble nitrogen compounds and for certain other variables determined in the above-mentioned experiments.

NOTATION

The following symbols will be used, the contents of various components of the leaf material being on a dry weight basis.

N, ammonia-nitrogen content

A_R, residual amino-nitrogen content

M, $2 \times$ amide-nitrogen content

P, protein-nitrogen content

S, reducing-sugar content

U, water content

pH, that of the expressed sap

R, respiration rate

P, probability that a given correlation or regression coefficient should arise, by random sampling, from an uncorrelated population

V, defined in Table I

¹ This investigation is one of a series financed co-operatively by the Carnegie Corporation of New York, the Australian Council for Scientific and Industrial Research, and the University of Adelaide.

THE AMMONIA-NITROGEN CONTENT

When N is plotted against the amount of nitrogen supplied externally it is found that the slope of the curve increases markedly with the latter quantity. The point at which the first very pronounced increase in N occurs is frequently also that at which U begins to fall rapidly; possibly the fall in U produced some change in the cells leading to increase in their permeability to ions or molecules.¹ Examination of the data shows that the large quantities of ammonia in the leaves of the plants with high nitrogen treatments entered from outside and did not arise from protein hydrolysis.

As was observed in paper I, the large values of N are not associated with increased pH of the expressed sap. Since the completion of the experiments, estimations of total organic-acid content of the dried leaf material have been carried out by the method of Pucher, Vickery, and Wakeman (1934);² the mean results of duplicate determinations are plotted in Figs. 2 and 6 for Experiments I and III, the two experiments where high values of N were obtained. It was found that the equivalent amount of organic acids present exceeds that of ammonia even with the highest nitrogen treatments, and this may account for the absence of increase in pH. Alternatively, of course, the ammonium ion could have been neutralized by the simultaneous uptake of anions.

THE RESIDUAL AMINO-NITROGEN CONTENT

Experiment I.

The drifts with time are illustrated in Fig. 1; the chief point of note in this figure is the tendency of the drifts in A_R to follow the direction of those in R . Fig. 2, illustrating the treatment effects, shows that A_R increases with N ; this was not revealed in Fig. 1, where the drifts of N are small. In equation (1), Table I, is given the regression of A_R on $\log N$ and R . The coefficient for $\log N$ is insignificant ($P = 0.1$), although omission of this term considerably decreases the goodness of fit as measured by V in the table (equation (3)); omission of the R term renders the $\log N$ coefficient significant, but decreases V (equation (2)). These phenomena are probably due to the fact that R is correlated with N (correlation coefficient = 0.70; $P < 0.01$), and consequently takes up a portion of the variance really attributable to N . At the same time it is probable that part of the variance of A_R is ascribable only to R ; in other words, there may be a real relationship between A_R and R . These relationships can be seen by examination of Fig. 7, where the data for A_R for the whole experiment are plotted against N .³ Substitution of N for $\log N$

¹ The decrease in U may have been caused, as suggested in paper I, by the effect of ammonium-ions or ammonia on the roots; cf. also Petrie (1937).

² These estimations were made by Miss J. E. Brooke.

³ Whenever in this paper the data for successive days are combined, it must be taken into account that any relationship among the variables could conceivably be obscured if the system was at different distances from a steady state on different days of the experiment, or even with different treatments.

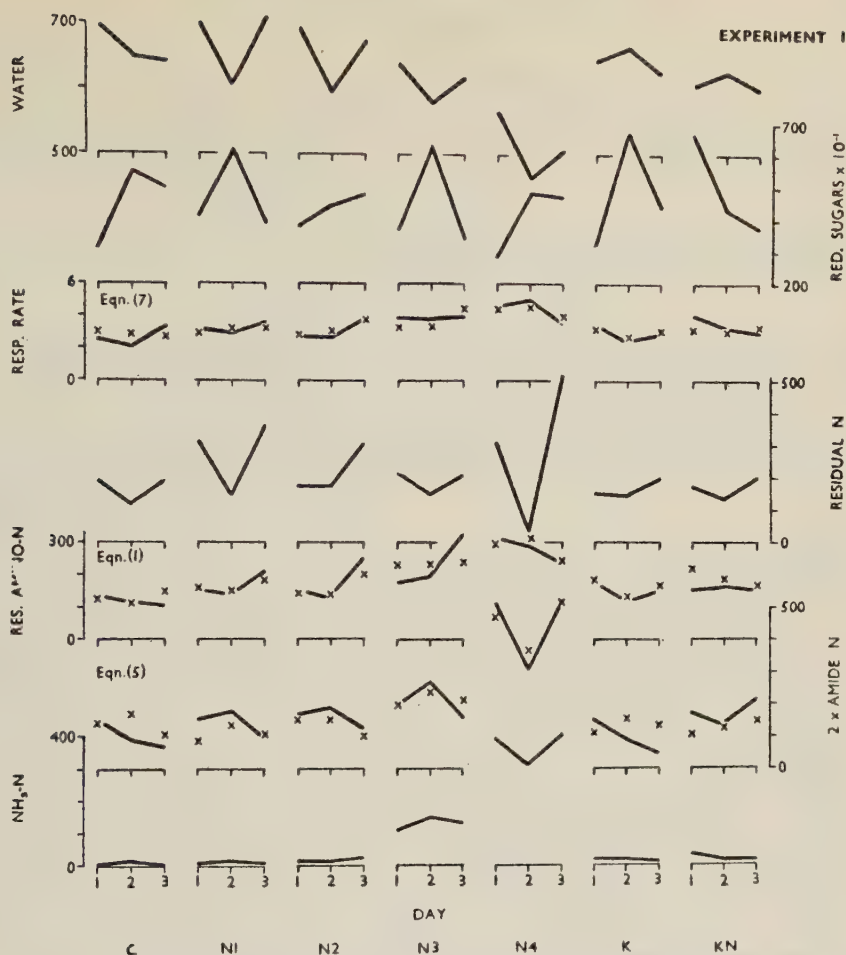


FIG. 1. Drifts with time in the contents of various components of the leaves, and in R , Experiment I. Values of R , A_R , and M calculated from the regression equations of Table I are also plotted, the equation number being given in each case. (From the data of Table IV paper I.)

Units (including all quantities expressed in this and subsequent figures):

Water: gm. associated with 100 gm. dry matter.

Organic acids: milli-equivalents per 100 gm. dry matter.

Reducing sugars: as mgm. glucose per 100 gm. dry matter (the values, not being significant to four figures, have been multiplied by 10^{-1}).

Respiration rate: mg. CO_2 per gm. per hour.

Nitrogen compounds: mg. per 100 gm. dry matter.

in equation (1), giving equation (4), results in a slight decrease in V ; there is thus a possibility that the A_R - N relation is represented by a curve concave to the N axis, rather than by a straight line. When terms in pH and U are added to the regression they are found to have insignificant coefficients and to decrease V ; this is also found when a U term is added to equation (2); but

TABLE I

Regression Equations

All the regressions are significant at or below the 5 per cent. point. Coefficients significant at or below the 5 per cent. point are given in **bold** figures, those not significant in *italic* figures.

NOTATION

V percentage of the variance of the dependent variable ascribable to the average effect of the independent variables; derived from the equation

$$V = 100 \left\{ 1 - \left(\frac{n-1}{n-p-1} \right) (1-R^2) \right\}$$

where n is the number of observations, p the number of independent variables, and R the multiple correlation coefficient of the dependent variable with the independent variables.

D.F. residual degrees of freedom.

$\sqrt{\text{res. var.}}$ square root of variance of dependent variable not accounted for by the regression.

The remaining symbols are as given in the list earlier in the paper.

The units are those given in the tables of paper I and in the legend to Fig. 1 of the present paper.

	V	D.F.	$\sqrt{\text{res. var.}}$
EXPERIMENT I.			
(1) $A_R = a + b_1 \log_{10} N + b_2 R$ $a = -42.5$ $b_1 = 37.3 \pm 21.27$ $b_2 = 52.3 \pm 18.85$	71	12	39.51
(2) $A_R = a + b \log_{10} N$ $a = 71.6$ $b = 79.1 \pm 10.43$	55	13	48.68
(3) $A_R = a + bR$	40	13	56.25
(4) $A_R = a + b_1 N + b_2 R$	66	12	42.42
(5) $M = a + b_1 N - b_2 R$ $a = 245.5$ $b_1 = 1.06 \pm 0.011$ $b_2 = 43.37 \pm 20.112$	90	12	42.26
(6) $M = a + bN$ $a = 96.7$ $b = 1.06 \pm 0.011$	87	13	49.53
(7) $R = a + b_1 A_R - b_2 U$ $a = 3.48$ $b_1 = 0.00785 \pm 0.001865$ $b_2 = 0.00253 \pm 0.000846$	68	12	0.45
EXPERIMENT II.			
(8) $A_R = a + b_1 \log_{10} N + b_2 U$ $a = -97.7$ $b_1 = 80.6 \pm 18.50$ $b_2 = 0.275 \pm 0.0733$	57	18*	22.13
(9) $A_R = a + b_1 N + b_2 U$	32	18*	27.43
(10) $R = a + b_1 A_R - b_2 U$ $a = 5.36$ $b_1 = 0.00559 \pm 0.004036$ $b_2 = 0.00592 \pm 0.002015$	26	18	0.550
(11) $R = a - bU$	22	19	0.565

* C, day 1, was omitted in calculating this regression as there is no value for A_R : N3, day 1, was also omitted, as was the case in the calculation of equations (5) and (6), Table VII, in paper I; this was done as there was some doubt as to the accuracy of the A_R value for this sample.

TABLE I (contd.)

EXPERIMENT III.	V	D.F.	$\sqrt{\text{res. var.}}$
(12) $A_R = a + b \log_{10} N$ $a = -23.0$ $b = 165.0 \pm 14.7$	90	13	45.20
(13) $A_R = a + b_1 \log_{10} N + b_2 U$ $a = -78.0$ $b_1 = 176.0 \pm 25.6$ $b_2 = 0.0994 \pm 0.227$	97	12	23.80
(14) $A_R = a + bS$ $a = -98.0$ $b = 79.2 \pm 7.30$	90	13	45.89
(15) $M = a + b \log_{10} N$ $a = -140.0$ $b = 324 \pm 29.00$	90	13	88.00

the high correlation between N and U (correlation coefficient = -0.82 ; $P < 0.01$) may obscure a possible relation of A_R to U .

Experiment II.

The time drifts are illustrated in Fig. 3: those for A_R are mostly small, and there is no suggestion of relation of A_R to R or to any other variables. Fig. 4 shows the relation to ammonium sulphate treatment and Fig. 5 that to asparagin treatment: on the whole, N , A_R , and R tend to increase with treatment. In equation (8) is given the regression of A_R on $\log N$ and U , and the calculated values are plotted in Figs. 3, 4, and 5; the data are also plotted as a whole in Fig. 8. The relation of A_R to N is given by curves concave to the N -axis, the concavity being particularly apparent if the individual points are joined for days 2 and 3; equation (9) shows that the fit to a linear equation is less good; but it is not suggested that the logarithmic function is the best fit, although it describes the concavity sufficiently well for present purposes.

It is noteworthy that there is a significant partial regression of A_R on U ; in this experiment U and N are unrelated (correlation coefficient = $+0.055$; $P = 0.8$), under which conditions perhaps the A_R - U relation is revealed. There is, however, ground for regarding this observation as not entirely conclusive. Examination of Fig. 8 shows that the significance of the relation of A_R to U rests on the data of day 4, which were obtained from plants of necessity placed in the cabinets a day later than those used for analysis on days 1-3; the day 4 data may not, therefore, be exactly comparable with the rest. Furthermore, the plants of $U = 214$ and $U = 245$ had dry weights significantly below the average (see Table II, paper I), which may also render the data for these plants incomparable with the rest.

No relation to R is apparent, as is found on addition of a term in this variable to the regression equation. The data may be insufficient to reveal a relationship to a third variable even if relationship existed: the goodness of fit in equation (8) is considerably less than it was for equation (1) for Experiment I, possibly in part because of the smaller range over which the values of N extend.

Examination of the calculated values for A_R does not suggest that the

EXPERIMENT I

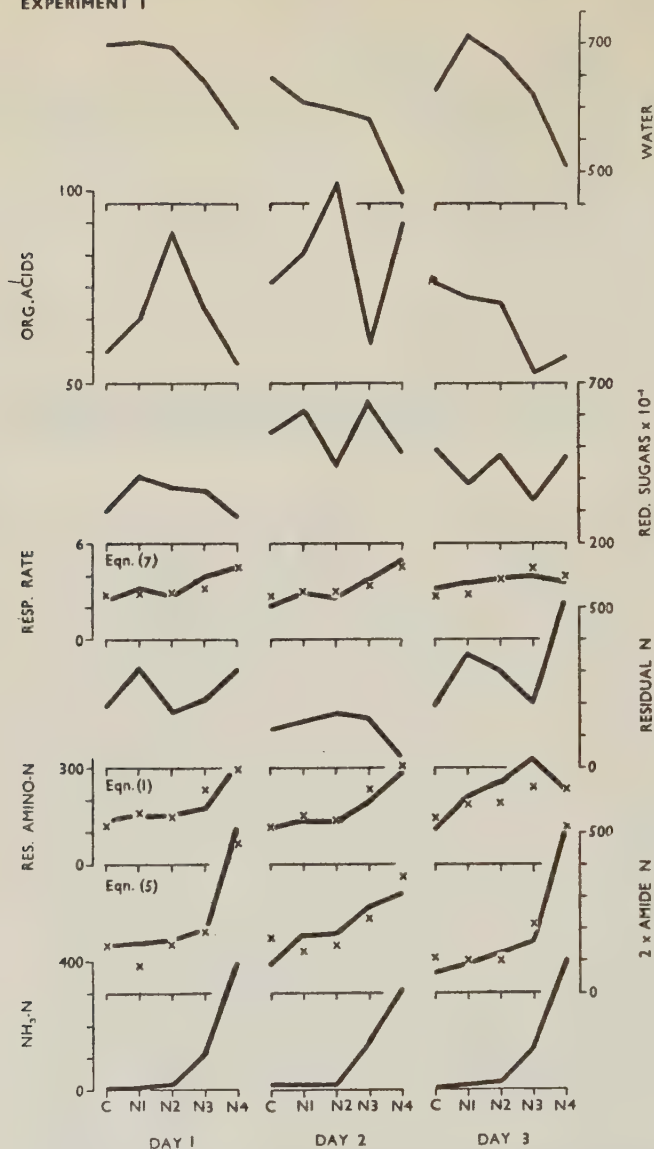


FIG. 2. Treatment effects on the contents of various components of the leaves, and on R , Experiment I. Values of R , A_R , and M calculated from the regression equations of Table I are also plotted, the equation number being given in each case. Units are as given in the legend to Fig. 1. (From data of Table IV, paper I.)

A_R - N relation is different with asparagin treatment from that with ammonium sulphate treatment; similarly, if the points for the ammonium sulphate treatments are joined separately for the individual days, those for the asparagin-treated plants do not depart appreciably from the curves so obtained.

Experiment III.

The time drifts in this experiment are slight and have not been considered worth illustrating, but Fig. 6 shows that A_R , N , and S all rise with treatment. In the regression equations of Table I, it is seen that 90 per cent. of the variance of A_R can be accounted for in terms of either $\log N$ or S , but when

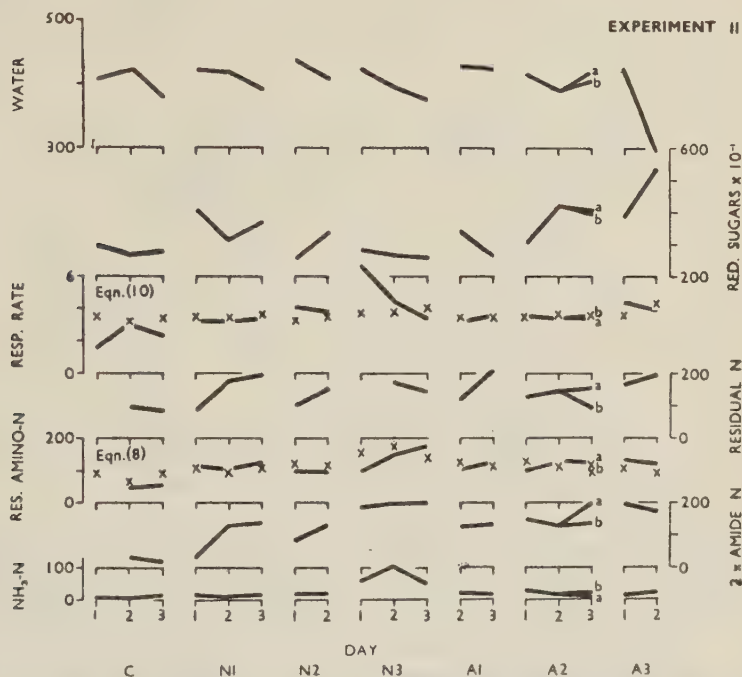


FIG. 3. Drifts with time in the content of various components of the leaves, and in R , Experiment II. Values of R and A_R calculated from the regression equation of Table I are also plotted, the equation number being given in each case. Units are as given in the legend to Fig. 1. (From data of Table V, paper I.)

both variables are included in the regression it is found that there is partial regression only with $\log N$; this is obviously the result of the correlation between N and S , which perhaps arises from the fact that both these variables increase with decrease in U . When a term in U is added to the regression it has an insignificant coefficient, but increases V (equation (13)); this is perhaps partly due to the high correlation between N and U (correlation coefficient = -0.96 ; $P < 0.01$); the standard error of the U coefficient is very high, and there is no evidence here of a real relation between A_R and U . In Fig. 9 the observed values of A_R are plotted against N , together with equation (12). That the goodness of fit is so great compared with that in the other experiments is probably mainly due to the wide range over which the values of the independent variable extend.

General conclusions.

It is known that amino-acids are liberated in the hydrolysis of proteins and are also synthesized from ammonia and certain carbon compounds probably formed in glycolysis. They can furthermore be deaminated to form

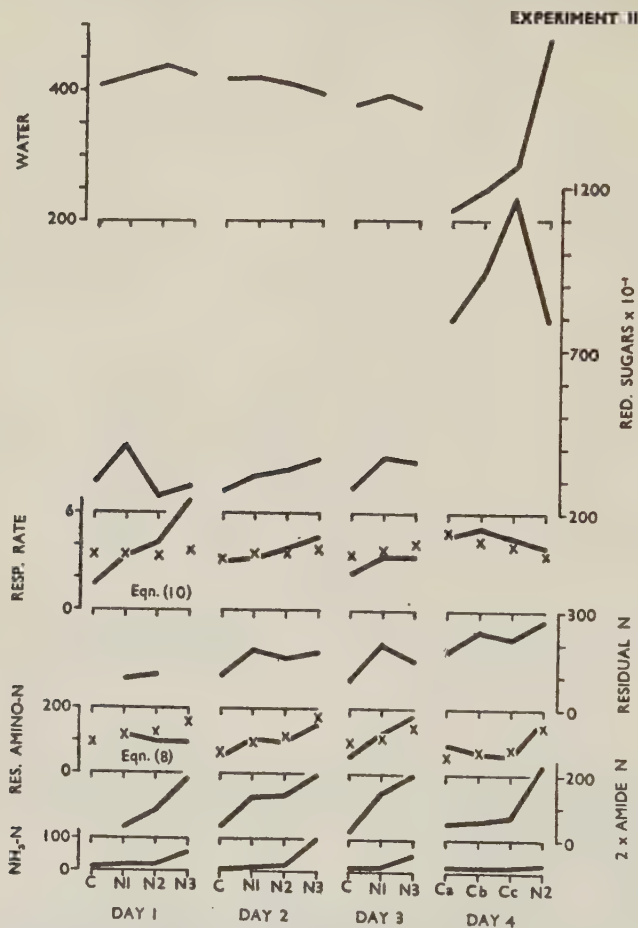


FIG. 4. Effect of ammonium sulphate and varying water-supply treatments on the contents of various components of the leaves and on R, Experiment II. Values of R and A_R calculated from the regression equations of Table I are also plotted, the equation number being given in each case. Units are as given in the legend to Fig. 1. (From data of Table V, paper I.)

ammonia and carbon residues.¹ It does not follow that degradation proceeds in the reverse direction to that of synthesis: in the process of oxidative deamination different carbon residues may result from those forming the reactants in synthesis, and the sole fate of such residues may be that of con-

¹ A schema for the reactions in nitrogen metabolism with which we are concerned was given in paper I; since that paper was written, two others have appeared—Gregory and Sen (1937) and McKee (1937)—in which there is further relevant discussion.

sumption in respiration. Nevertheless, if a steady state or an approximation thereto was attained in the experiments, we may expect that the concentration of amino-acids would be related to that of both of their precursors, although,

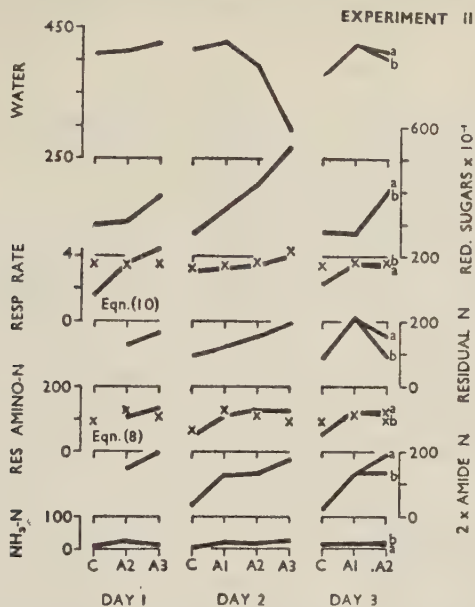


FIG. 5. Effects of asparagin treatment on the contents of various components of the leaves and on R , Experiment II. Values of R and A_R calculated from the regression equations of Table I are also plotted, the equation number being given in each case. On day 3, R_{calc} has the same value for both treatments A2a and A2b. Units are as given in the legend to Fig. 1. (From data of Table V, paper I.)

of course, probably a number of different amino-acids are synthesized from different carbon compounds.

In all three experiments the curve relating A_R to N is concave to the N axis, and it is proposed to consider possible explanations for this fact; however, reference must first be made again to the existence of some evidence, in the data of Experiment II, for a relation of A_R to U . In Experiments I and III the high correlation between N and U may obscure a similar relation of A_R to U ; if such a relation existed, it would imply that with constant U the concavity of the A_R - N curves should be less than that found, although the results of Experiment II indicate that they are still concave even with constant U . From this fact alone it would be expected, employing reasoning similar to that set out in paper I for the P - A relation, that there should be a relation of A_R to U .

The concavity, then, is not accounted for by any relation of A_R to U . Another explanation that presents itself can also be excluded. It could be suggested that the property is due to the system in the different plants being at different distances from a steady state (drifting or constant), and that at

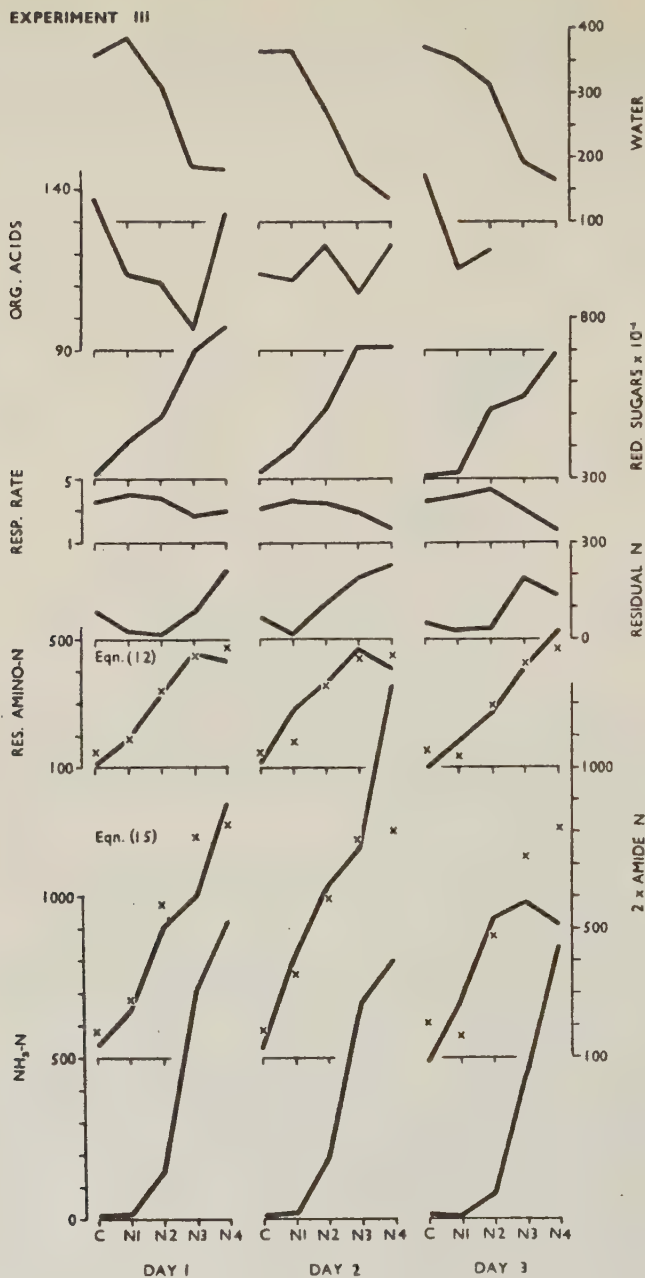


FIG. 6. Treatment effects on the contents of various components of the leaves, and in R, Experiment III. Values of A_R and M calculated from the regression equations of Table I are also plotted, the equation number being given in each case. The units are as in the legend to Fig. 1. (From data of Table VI, paper I.)

such state the curve would be of different form. More specifically, it could be suggested that, where N is high, the amino-acid content had not reached a steady state, whereas a closer approach to such state had been attained where N is low. A survey of the drift graphs, however, reveals that, when N is

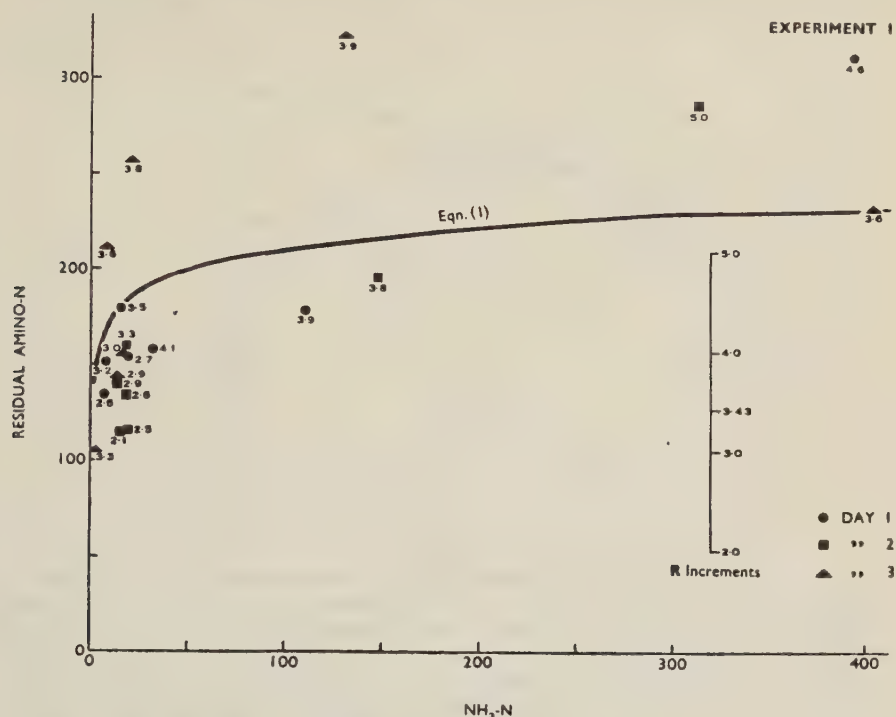


FIG. 7. A_R plotted against N , Experiment I. The corresponding value of R is given for each point. The curve is that of equation (1), Table I, for the mean value of R ($= 3.43$). The scale of R increments shows the distance that, according to the equation, has to be added to, or subtracted from, the ordinate of any point on the curve to give the value of A_R corresponding to a point with the same value of N but with a value of R above or below 3.43. Units are as given in the legend to Fig. 1. (From data of Table IV, paper I.)

high, A_R is frequently falling with time rather than rising; the suggestion is therefore unjustified, unless there is a very considerable lag between change in content of ammonia nitrogen and the commencement of change in content of amino-nitrogen in the same direction.

Two possible explanations for the concavity may now be considered. In the first place, the concentration of the carbon compounds may have acted as a limiting factor, as would be the case, for instance, if these compounds were formed at a constant limiting rate, or at a rate that falls with rise in N . There is no information as to the amounts present of the non-nitrogenous precursors of amino-acids; it is possible that these are organic acids, but the total organic-acid content shows no relation to A_R , or in fact to any variables measured.

A second explanation may be that, when a steady state is reached, the concentration of amino-acids is maintained at a value higher than that of equilibrium by the expenditure of metabolic energy; in this case the form of the relation between amino-nitrogen content and the content of its precursors

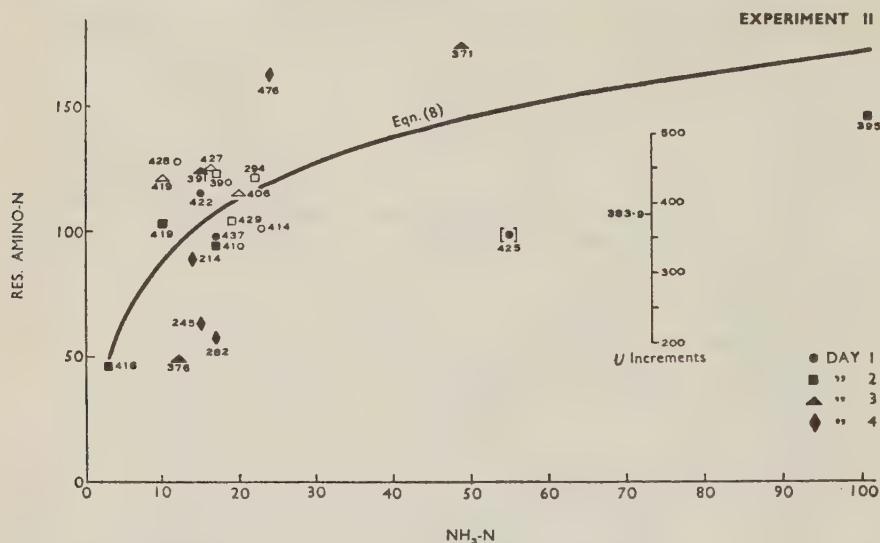


FIG. 8. A_R plotted against N , Experiment II. The corresponding value of U is given for each point, and the diacritic symbols marking the points corresponding to the asparagin treatments have white centres. The curve is that of equation (8), Table I, for the mean value of U ($= 383.9$). The value for N_3 , day 1, was omitted in calculating the regression, and the corresponding point is enclosed in brackets. The scale of U increments shows the distance that, according to the equation, has to be added to, or subtracted from, the ordinate of any point on the curve to give the value of A_R corresponding to a point with the same value of N but with a value of U above or below 383.9. Units are as given in the legend to Fig. 1. (From data of Table V, paper I.)

would be a function of the rate of energy release and could be of the concave form observed. It is of interest in this connexion that, in Experiment I, A_R increases with R : a possible explanation is that both R and amino-acid concentration increase with increase in concentration of glycolysis products;¹ it is, however, also a possibility that, if the concentration of amino-acids is maintained at a non-equilibrium value by expenditure of energy, the amount of energy expended in this direction may increase with increase in R ,² so that the concentration of amino-acids, and hence A_R , would increase with R .

It should perhaps be noted here that Richardson (1934) has shown that the van Slyke method does not give quantitative estimates of certain amino-acids; whether this could lead to a distortion of the A_R - N curve it is impossible to say.

¹ It should be noted here that the variation in R can be partly accounted for in terms of the variation in A_R and U .

² As pointed out elsewhere (Petrie, 1933), this type of relationship is not inevitable: it depends on the manner in which the various oxidative mechanisms of the cell are interlinked.

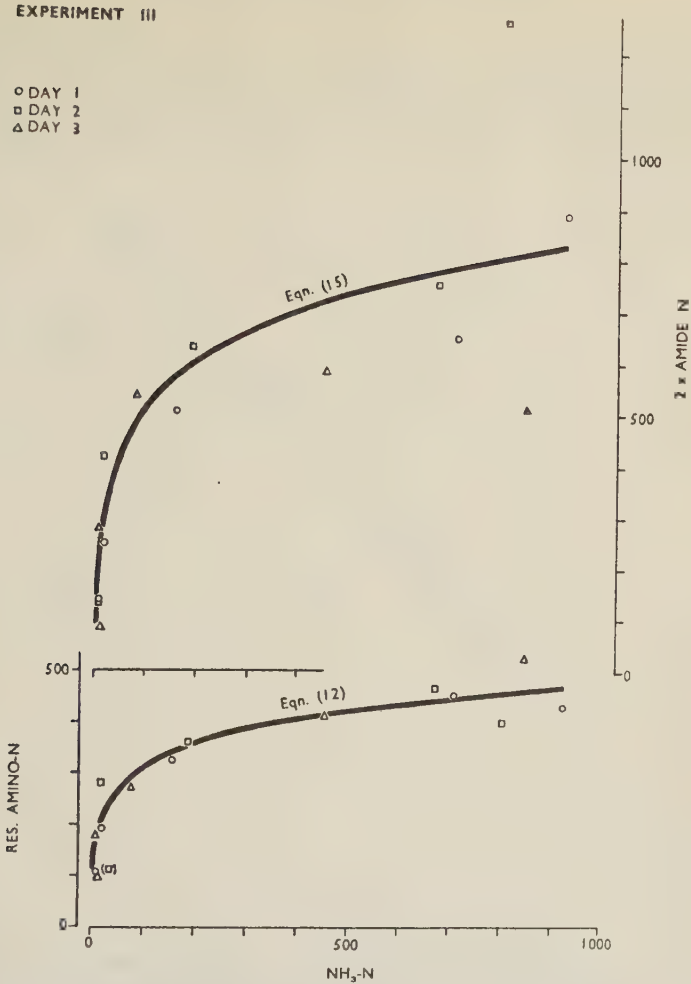


FIG. 9. A_R and M plotted against N , Experiment III. The curves are those of equations (12) and (15), Table I. Units are as given in the legend to Fig. 1. (From data of Table VI, paper I.)

THE AMIDE-NITROGEN CONTENT

Experiment I.

The drift with time is illustrated in Fig. 1; whereas the drifts in A_R tend to follow those in R , the drifts in M tend to be in the reverse direction. Fig. 2 reveals M also as increasing with N , on the whole more rapidly than A_R . Equation (5), Table I, gives the regression of M on N and R ; inclusion of a term in R slightly improves the fit, although the coefficient just fails to be significant at the 5 per cent. point ($P = 0.052$); substitution of $\log N$ for N , whether or not the R term is included, considerably decreases the goodness of fit; the linearity of the relationship is also shown in Fig. 10, where the M

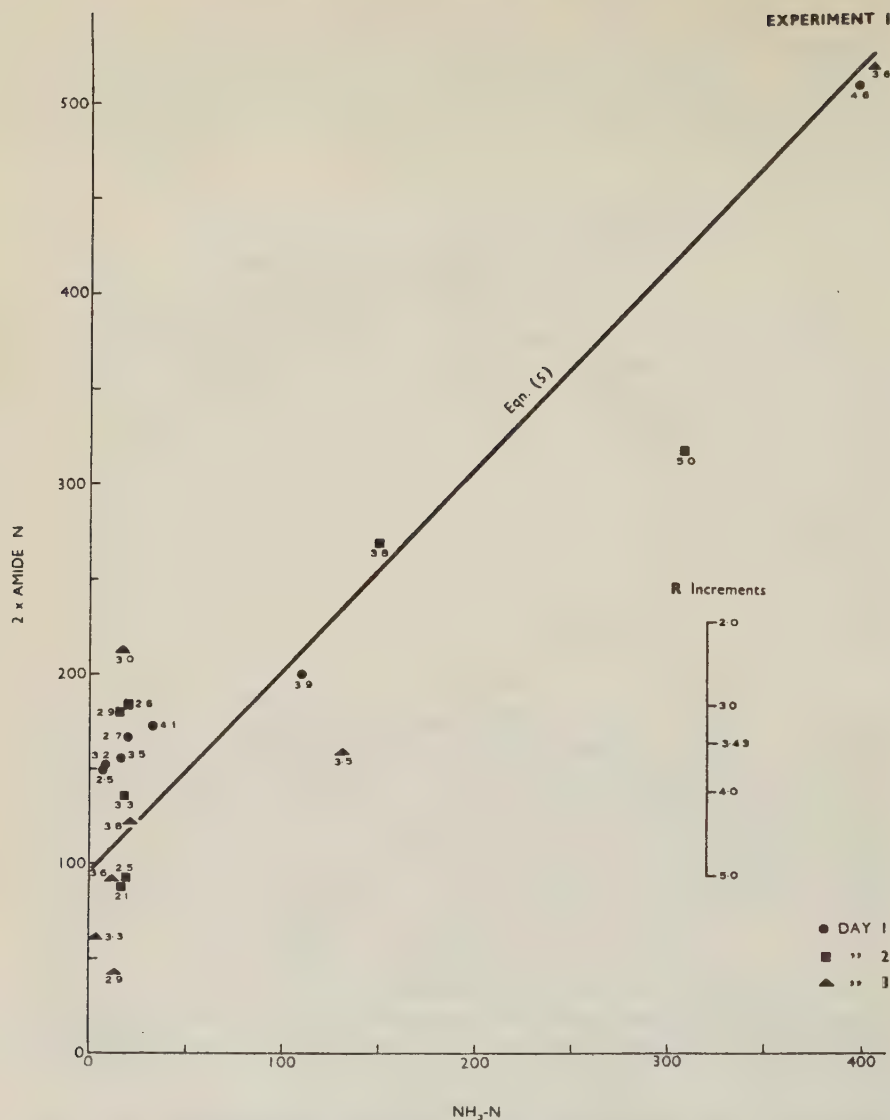


FIG. 10. M plotted against N , Experiment I. The corresponding value of R is given for each point. The line drawn through the points is that of equation (5), Table I, for the mean value of R ($= 3.43$). The scale of R increments shows the distance that, according to the equation, has to be added to, or subtracted from, the ordinate of any point on the curve to give the value of M corresponding to a point with the same value of N but with a value of R above or below 3.43. Units are as given in the legend to Fig. 1. (From data of Table IV, paper I.)

data are plotted against N and the regression line of equation (5) is also drawn. When terms in pH and U are added to the regression they are found to have insignificant coefficients and to decrease V .

Experiment II.

Fig. 3 illustrates the time drifts; no definite correlation is suggested between the drifts in M and those in any other variable. Figs 4 and 5 show that M increases with N ; this is also shown in Fig. 11, where M is plotted against N for each day. Statistical and graphical examination of the data does not reveal

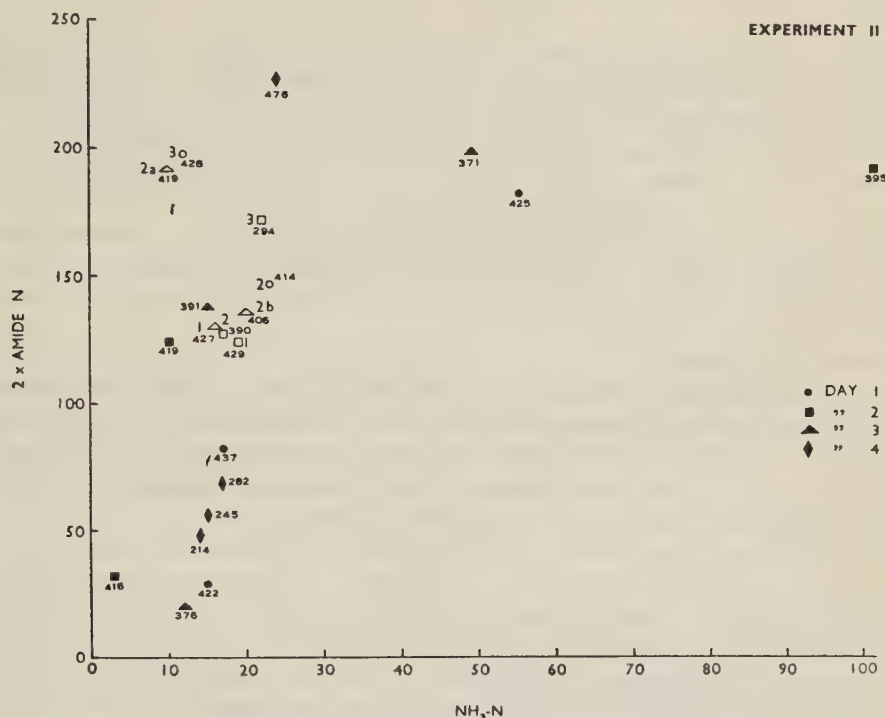


FIG. 11. M plotted against N , Experiment II. The corresponding value of U is given for each point. The diacritic symbols marking the points corresponding to the asparagin treatments have white centres: the treatment designations are given against these symbols in the larger figures, the A's being omitted; e.g. treatment A2 is designated 2, &c. For reasons given in the text, no curve is drawn through the points. Units are as given in the legend to Fig. 1. (From data of Table V, paper I.)

any partial relation of M to R or U ; we have not, therefore, given a regression equation for the relation of M to N alone, since the curvature is such that it cannot be accurately expressed by a simple regression function. Disregarding the data for the asparagin treatments, it is evident that the points fall closely on a curve concave to the N axis, with the exception of one point on day 4, which has the high U value of 476; the data, however, are not complete enough to define the curve accurately, especially in the region of maximum curvature.

There is a suggestion that, with the asparagin treatments, where M is large (viz. in A3, day 1; in A2a, day 3; and perhaps to some extent in A3, day 2, where M is not so large), the points tend to lie above the M - N curve:

where M is comparatively small, the points conform to the relation indicated by the points for the ammonium sulphate treatments. This would suggest a considerable disturbance of the steady state with high asparagin treatment; but the suggestion is not a strong one on account of the general scatter of the points.

Experiment III.

The time drifts in this experiment are slight and reveal no distinctive features. Figs. 6 and 9 show again that M increases with N , and the M - N relation is again given by a curve concave to the N axis. The regression given as equation (15), Table I, and the calculated points plotted in Fig. 6, show that the variance in M can be largely accounted for by variation in N . There is also a correlation between M and S , attributable, as in the case of the amino-acid data, to the correlation between $\log N$ and S . The addition of a term in U to the regression gives an insignificant coefficient and decreases V .

General conclusions.

Amides are probably liberated in protein hydrolysis, but their main source is that of synthesis from carbon compounds and ammonia. The picture is thus somewhat similar to that suggested for amino-acid synthesis, and again we are probably not concerned here with a single compound, but at least with both asparagin and glutamin (see Schwab, 1936).

The data as a whole show that a large amount of the variance in M can be accounted for in terms of the variation in N . Except in Experiment I, the M - N curve is concave to the N axis; and the slope at any point is usually greater than that of the corresponding A_R - N curve. Similar suggestions could be made as to the reason for this concavity as were made for the amino-acid data; it is evident again, on examination of the drift data of Experiment II, that concavity is not due to the high M values being most removed from those characteristic of the steady state.

No significant partial regression on any variable other than N can be found. The insignificant one on R in Experiment I indicates no real relationship to respiration rate, especially as there is no further suggestion of it in the other experiments. It is worth noting, however, that Fife and Frampton (1935) found that, when sugar-beet leaves are exposed to high concentrations of carbon dioxide, amides are reversibly broken down giving free ammonia which increases the pH of the sap;¹ this suggests that the formation of amides may in some circumstances be affected by the carbon dioxide produced in respiration. As in the case of A_R , there is no relation of M to U or sap pH. A relation to U may again have been obscured by the correlation between N and U in Experiments I and III, and it might be concluded from Fig. 11 that, in Experiment II, such relation could have been obscured by the

¹ In passing, it is interesting to note that Thornton (1933) also found that, in presence of oxygen, carbon dioxide made sap more alkaline.

scatter in the data; perhaps, therefore, until further data are available, no great significance should be attached to the failure to reveal a relation between M and U . In contrast to the present finding, Kultscher (1932) and Garber (1935) concluded that the M - N relation is largely determined by the sap pH, the 'equilibrium', according to Kultscher, being shifted more towards amide the higher the pH.

The increase of N in the leaves of asparagin-treated plants as compared with untreated shows that break-down of asparagin occurred. In fact, it is evident that asparagin was converted into amino-acids, probably partly via ammonia; there is no suggestion in Fig. 8, however, that aspartic acid accumulated so much as to deflect the A_R - N relationship from that occurring with the other treatments.

THE RESIDUAL-NITROGEN CONTENT

The residual-nitrogen content values have been included in the graphs for completeness although they do not reveal any definite relationships to other quantities. There is an occasional tendency for the residual nitrogen content to rise with N , and in Fig. 1 the time drifts tend to follow those of U and P , except with treatments K and KN. Residual-nitrogen content values, of course, are subject to considerable inaccuracy inasmuch as they are obtained by difference, and in any case the residual-nitrogen comprises several dissimilar chemical entities.

THE RESPIRATION RATE AND THE REDUCING-SUGAR CONTENT

Experiment I.

The R drifts tend to follow the direction of those in a number of other variables, viz. P , A_R , U , and pH, as is seen from Fig. 1 and from Fig. 1 of paper I; Fig. 2 shows, however, that R is affected by treatment in the opposite direction to U . In Fig. 12 the data are combined and R is plotted against U . The negative correlation is again apparent; examination of the combined data also shows, however, that R is related either to A_R or N . The regression of R on A_R and U is given in equation (7), Table I, and the regression line is drawn in Fig. 12 for the mean value of A_R ; the selection of A_R rather than N is arbitrary, and, as these two variables are highly correlated, equally good relations could probably be found between either of them and R .

The reducing-sugar content tends to drift with time in the opposite direction to R , but otherwise shows no relationships with other variables.

Experiment II.

The drifts with time in R (Fig. 3) tend to follow those in U , whereas the drifts in S tend to be in the reverse direction. In the graphs showing treatment effects (Figs. 4 and 5) there is a frequent tendency for R and S to increase together, but otherwise no distinctive features are apparent. In Fig. 13 R is plotted against U : excluding one point of high R value enclosed

in brackets a negative correlation is again evident. In equations (10) and (11) are given the regressions of R on U and A_R , and on U alone; the partial regression on A_R is insignificant, although inclusion of the A_R term slightly improves the fit of the equation. The data for N_3 , day 1 (the point referred

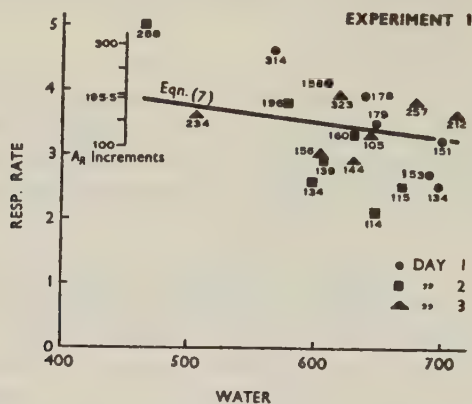


FIG. 12. R plotted against U , Experiment I. The corresponding value of A_R is given for each point. The line drawn through the points is that of equation (7), Table I, for the mean value of A_R ($= 195.5$). The scale of A_R increments shows the distance that, according to the equation, has to be added to, or subtracted from, the ordinate of any point on the line to give the value of R corresponding to a point with the same value of U but with a value of A_R above or below 195.5. Units are as given in the legend to Fig. 1. (From data of Table IV, paper I.)

to above), were omitted in calculating the regression, as there is some doubt concerning the accuracy of the observed value for A_R ($= 98$); the value calculated from equation (8) is 157, which accords better with the suggested relation between R and A_R . The unaccountable variance in R is high.

Experiment III.

The drifts with time are too small to allow conclusions to be drawn. Treatment effects are shown in Fig. 6: S rises with treatment, whereas R rises at first and then falls. In Fig. 14 R is plotted against U : here there is very little suggestion of a relation of R to A_R ; as U falls to a value in the region of 300, R shows little general change; with further fall in U , R also falls. The high correlation between A_R and U may have obscured any relation of R to A_R . As has already been mentioned, this experiment also shows a high correlation between S and N .

General conclusions.

The data discussed above show that, down to a certain level, fall in U is associated with rise in R : beyond this level further fall in U evidently causes R to fall again. This is in conformity with results found by other workers. Bouillenne-Walrand (1926) dried Brassica roots in darkness; the initial water content was 1,200–2,000 gm. per 100 gm. dry matter; respiration

rate rose with progressive desiccation but declined sharply again when the water content was reduced to about 300 gm. per 100 gm. dry matter. Smith (1915) deprived various plant organs of varying proportions of water. With a loss of up to 30 per cent. of the initial water content, respiration rate was

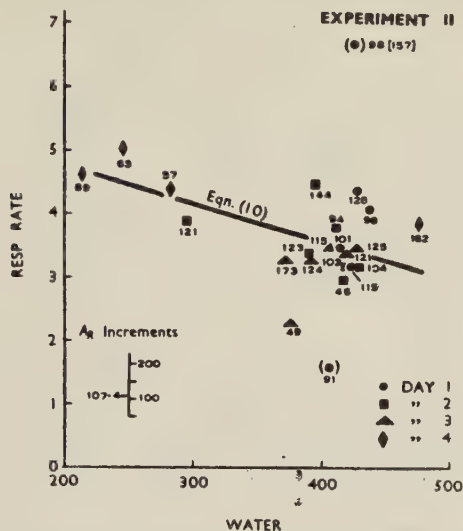


FIG. 13. R plotted against U , Experiment II. The corresponding value of A_R is given for each point. The line drawn through the points is that of equation (10), Table I, for the mean value of A_R ($= 107.4$). The point for C, day 1, is enclosed in brackets and marked 91; this point was not included in calculating the regression, as there is no observed value for A_R : 91 is the value calculated from equation (8). The point for N3, day 1 ($A_R = 98$) is also enclosed in brackets, as it was omitted from the calculation of the regression: 157 is the calculated value of A_R . The scale of A_R increments shows the distance that, according to the equation, has to be added to, or subtracted from, the ordinate of any point on the line to give the value of R corresponding to a point with the same value of U but with a value of A_R above or below 107.4. Units are as in the legend to Fig. 1. (From data of Table V, paper I.)

increased; further water loss up to 50–60 per cent. of the initial content led to no further effect, and thereafter respiration rate decreased again. Smith suggests that the phenomenon is due to an effect of water content on enzyme activity. Similar results were found in potato tubers by Palladin and Sheloumova (1918). Collorio (1928) found that wilting plants had a greater respiration rate than the controls.

It is not proposed to suggest any interpretation for this relationship of R to U in the present paper. The interpretation must take into consideration the effect of water content on the content of various carbohydrate substances in the leaves, and the present reducing-sugar content data are inadequate for such consideration. Recent investigations in these laboratories by Mr. G. L. Amos have shown that, in experiments such as those discussed, a considerable amount of non-sugar reducing substances is present in the leaves, so the data given here may not be reliable indications of the true hexose contents.

In Experiment I, R significantly increases with A_R . Such a relation has also been found by Spoehr and McGee (1923), Genevois (1927), Schwabe (1932), and Gregory and Sen (1937). Spoehr and McGee consider the possibility of an accelerating influence of amino-acids on diastatic activity;

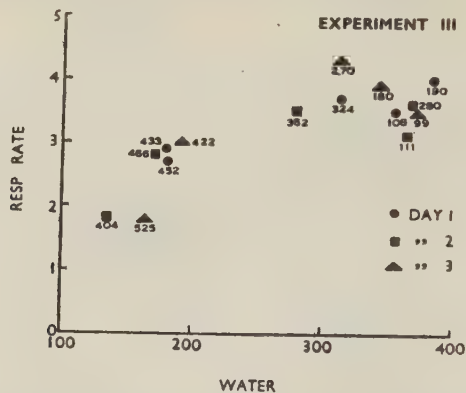


FIG. 14. R plotted against U , Experiment III. The corresponding value of A_R is given for each point. For reasons given in the text, no curve is drawn through the points. Units are as given in Fig. 1. (From data of Table VI, paper I.)

and Hartt (1934) showed that asparagin greatly increases the hydrolytic activity of amylase. The increase in R may also be due to the deamination of amino-acids, which will be more rapid the greater the amino-acid concentration: the non-nitrogenous fraction of the amino-acids may afford a substrate for respiration. This view has been put forward by Gregory and Sen. Hamner (1936) has also shown an increase in respiration rate by the addition of nitrate to plants low in nitrogen but high in carbohydrate; he considers that this is probably due to elaboration of amino-acids and their subsequent effect on respiration rate.

SUMMARY

The relation in the leaves of grasses among the contents of soluble nitrogen compounds and of water and the respiration rate have been studied under constancy of certain environmental factors. The grasses used were *Phalaris tuberosa* L. and *Lolium multiflorum* Lam. The following conclusions were drawn, and there is ground for believing that these apply to the steady state, although such state was not necessarily attained in the course of the experiments:

1. Increase of ammonia-nitrogen content did not increase the pH of the expressed sap, possibly because the equivalent amount of organic acids present always exceeded that of ammonia-nitrogen.

2. The curve relating the content of residual amino-nitrogen to the ammonia-nitrogen content is concave to the ammonia-nitrogen content axis; this concavity might be due to the content of non-nitrogenous precursors of

the amino-acids acting as a limiting factor, or it might be due to the maintenance of a state of non-equilibrium between amino-nitrogen and ammonia-nitrogen as a result of the expenditure of energy. In one experiment residual amino-nitrogen content was also found to increase with increase in water content, and in another experiment with increase in the respiration rate, but neither of these relationships can be regarded as definitely established, since they were not revealed in all the experiments.

3. The curve relating amide-nitrogen to ammonia-nitrogen content in two cases is also concave to the ammonia-nitrogen content axis; no significant relation could be found between amide-nitrogen content and any variables other than ammonia-nitrogen.

4. Respiration rate rises as the water content falls to a certain level; further fall in water content causes respiration rate to fall. In one experiment also respiration rate significantly increased with increase in amino-acid content.

The authors are indebted to Mr. G. E. Briggs, F.R.S., of St. John's College, Cambridge, for his kindness in discussing the data with them prior to the writing of this paper, and in making certain valuable suggestions as to the manner of treating them.

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The Development of the Flowers from the Curd of Broccoli (*Brassica oleracea botrytis*)

BY

S. O. S. DARK

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With Plate XXIX

THE structure of the head or curd of the broccoli and cauliflower (*Brassica oleracea botrytis*) appears in the literature to have been misunderstood.

Masters (1849) says: 'The cauliflower and broccoli afford familiar illustrations of the hypertrophy of the flower stalk accompanied by a corresponding defective development of the flowers' (p. 421). According to Worsdell (1915), '... the whole inflorescence becomes greatly hypertrophied, involving very copious branching of the pedicellar system, and in which the flowers are completely suppressed, has given rise to the variety of the cabbage known as the cauliflower . . .' (vol. ii, p. 40), and 'Examples are afforded by the cauliflower and broccoli, in which, owing to excessive hypertrophy of the pedicels, the flowers are not developed, or only a vestigial calyx' (p. 248).

From this it would appear that the curd has developed into a purely vegetative organ. Actually the whole inflorescence is hypertrophied, but the flowers are not defective, the curd being merely an intermediate stage in the development of the flowers and seed.

After the curd has developed it is rapidly disrupted by the elongation of the main stalks to varying degrees. Plate XXIX, Figs 1-6, show successive stages in the further development of one of these pieces of the disrupted curd.

Fig. 1. 1st day, the masses of white hypertrophied flowers are separating by the elongation of the secondary stalks.

Fig. 2. 3rd day, the stalks are elongating further and losing their fleshy character. The white curd is developing pigment (in the centre at 2 o'clock), while (at 10 o'clock) pigment has been developed and the hypertrophied bud tissue has begun growing out into buds.

Fig. 3. 6th day, the same processes are continuing and the buds in the centre mass have now differentiated their sepals.

Fig. 4. 8th day, the minor branches are continuing to elongate and the pedicels of the buds are lengthening. At the right-hand side is a partially differentiated mass of buds that is beginning to abort, probably due to starvation, as it did not appear to have suffered any injury.

Fig. 5. 17th day, here the buds are nearly ready to burst. The aborted

portion of the curd is seen as a dried up mass at the bottom right-hand side.

Fig. 6. 24th day, all stages of flowering from unopened buds to the developing fruits are here seen.

A certain percentage of the curd will probably always abort through starvation, as the enormous mass of rapidly growing tissue must throw a great strain on the channels of food and water translocation. Apart from this portion, the whole curd should be capable of developing into flowers. In practice this does not occur as the curd is extremely susceptible to injury by damp, cold, insects, or bruising, resulting in the abortion of the affected portion. Under field conditions most of the curd loss is due to frost injury followed by an attack by *Botrytis* on the injured portion, and the fungus sometimes spreads to such an extent that the entire plant rots away.

SUMMARY

The growth of the flowers from the curd of broccoli is described and illustrated.

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EXPLANATION OF PLATE XXIX

Illustrating Dr. S. O. S. Dark's paper on 'The Development of the Flowers from the Curd of Broccoli (*Brassica oleracea botrytis*)'.

FIGS. 1-6. Untouched photographs of the same piece of broccoli curd at successive stages of growth. Figs. 1-4 are approximately natural size, Figs. 5-6 are approximately half natural size. For description see the text.



1



2



3



4



5



6

DARK - CURD OF BROCCOLI.

Huth, Stubbs X, Kent,

Studies in Vernalisation of Cereals

III. The Use of Anaerobic Conditions in the Analysis of the Vernalising Effect of Low Temperature during Germination

BY

F. G. GREGORY

AND

O. N. PURVIS

(From the Research Institute of Plant Physiology, Imperial College of Science and Technology, London)

With Plate XXX and three Figures in the Text

INTRODUCTION

MAXIMOV (1934), in discussing the theoretical significance of vernalisation, concluded from the data of Vasiliev (1934) that when the time of low-temperature treatment is included with the length of the vegetative period, the time elapsing before ear emergence is then no shorter in vernalised than in unvernalsed plants. Vasiliev himself claimed that this was so only over a limited range of durations and methods of treatment, and in a previous paper (Purvis and Gregory, 1937) a simple explanation was advanced to account for this result. Nevertheless, the present authors at that time accepted the statement of Maximov, and sought for a method, other than low temperature, to inhibit germination, and thus to prolong the period of germination. In this way it was hoped to separate the specific vernalisation effect of low temperature from that of mere inhibition of germination.

The method adopted was to use anaerobic conditions and high concentrations of CO_2 . The development of this technique made possible a successful experimental demonstration of a quantitative reversal of the vernalising effect of low by higher temperatures, as well as the complete inhibition of the low-temperature effect in the absence of oxygen. A preliminary account of these experiments has already appeared elsewhere (Gregory and Purvis, 1936, 1938).

Effects of completely anaerobic conditions on germination.

That cereal grains in a dry condition may be kept viable for long periods in an atmosphere devoid of oxygen has long been known. Van Tieghem and Bonnier (1882) showed that after two years' storage of dry grain of wheat at air temperature the germination was the same whether kept in presence or

absence of air. Filter and Laschke (1909) showed that this was no longer true when the grain was kept at 30° C., for then absence of air greatly reduced germinating power. Becquerel (1906) showed that no decrease in germination followed storage of wheat in a dry condition in nitrogen for one year.

In imbibed grains a rapid loss of germinating power takes place under anaerobic conditions as Dude (1903) showed with rye. Germination after

fifteen days was reduced 50 per cent., while fifty days' treatment killed all the grain.

The preliminary experiments in this investigation consisted in keeping winter rye grains in flasks completely filled with boiled water. Before treatment germination was 90 per cent., after three weeks in the dark this was reduced to 50 per cent., while in the light in the same time a further reduction to 20 per cent. resulted. In order to restrict as far as possible the leaching



TEXT-FIG. 1. Apparatus used for imbibing sterile grain with known volume of water under anaerobic conditions. For details see text.

of materials from the imbibed grains by the presence of large volumes of water, a method was devised whereby the grain was provided, in an atmosphere completely free of oxygen, with the exact amount of water for imbibition with no excess. For this purpose V-shaped glass tubes were made, sealed at the ends, and provided with two side arms through which gas could be passed, as shown in Text-fig. 1. Dry sterilized grain was placed in one limb, and the requisite amount of sterile tap-water was introduced into the other limb by means of a special pipette designed to deliver measured volumes of sterile liquid. It was found by preliminary trial that 2 gm. of wheat were fully imbibed by 1–1.5 c.c. of water, and rather less was needed for rye of the same weight. Before use the tubes were sterilized, and plugged with sterile cotton-wool in the side arms. After introducing the weighed quantity of sterilized seed the side arms were slightly drawn out, to facilitate sealing later, and water added to the other limb. By means of the side arms the tubes were connected in series and pure nitrogen passed for thirty hours. While still connected to the nitrogen supply the tubes were sealed off with a blow-pipe and stored, the grain being kept apart from the water. Imbibition of the grain was begun by tilting the tubes so that the water flowed over into the limb containing the dry grain. In the first trial exposures of 4, 9, 14, and 21 days were given at room temperature, while other tubes were kept for six weeks at 1° C. On opening the tubes the moist grain was placed in open dishes and set to germinate in the dark. In all cases the total percentage germination was equal to that of the controls, as seen in Table I.

The rate of germination varied with the treatment, 50 per cent. germination

occurring most rapidly after four days in nitrogen, but longer periods reduced subsequent germination rate. In this experiment later growth was not studied.

A second experiment was begun in the spring of 1935, and the plants were later grown to maturity in normal summer days. Both wheat (var. *Lutescens*

TABLE I

Germination of Winter Wheat (var. Lutescens 1060/10) after Exposure of Imbibed Grains to Pure Nitrogen

Days exposed to N ₂ .	0.	4.	9.	14.	21.	42 days at 1° C.
Percentage germination	94	100	94	96	95	94
Days to reach 50% germination	4.0	2.8	—	4.8	5.5	—

1060/10) and rye (var. *Petkus* winter) were used in this experiment (5 gm. seed 170 grains wheat, 155 rye per tube: water added for wheat 3 c.c.; for rye 2.5 c.c. per tube). The length of exposure to nitrogen was also increased, and the effect of temperature and light tested. Three tubes were used for each treatment. The controls were treated in the same way except that the water was kept apart from the grain. The treatments and results of germination are given in Tables II and III.

TABLE II

Germination of Winter Wheat (var. Lutescens 1060/10) after Exposure of Imbibed Grain to Pure Nitrogen at 20° C.

Weeks exposed to nitrogen.	0.	1.	2.	3.	5.	7.	9.	12.
Dark 20° C.	66	40	13	4	0	0	0	0
Light 20° C.	—	—	—	0.2	0	—	0	—
Dark 1° C.	—	—	—	—	—	—	20	7

TABLE III

Germination of Rye (Petkus) after Exposure of Imbibed Grain to Pure Nitrogen at 20° C.

Weeks exposed to N ₂ .	0.	1.	2.	3.	4.	5.	6 weeks at 1° C.
Petkus spring rye	87	77	49	7	0	0	51
Petkus winter rye	69	—	—	25	0	0	—

At 20° C. anaerobic treatment cannot be prolonged beyond three weeks, but at 1° C. exposures up to twelve weeks are not completely lethal. Even after storage at 1° C. on opening the tubes considerable volumes of gas escaped, mostly nitrogen and carbon dioxide, but a faint odour of ripe fruit was noticeable. No evidence of decay was present. In external appearance the seed was quite normal even after the longest exposures, but the endosperm was soft and glutinous, and in some cases had ruptured the seed coat. The embryos were turgid, white, and apparently undamaged. During the first day in air the lethal effect of the anaerobic conditions were manifested by

blackening and death of the grains. A curious feature was the appearance of a black ring in the aleurone layer in many of the wheat grains surrounding the embryo. Abnormalities in the seedlings were frequent. In a number of cases complete inhibition of root formation was noted, stunting of the roots was frequent, and the first leaves were often twisted and malformed. These abnormalities disappeared in the surviving seeds, which later became normal after the leaves had become green, which suggests a destruction or inhibition of growth substances during anaerobic germination, these later being formed or activated in the green leaves. A further experiment with spring and winter rye confirmed these results.

It was thought possible, in view of the apparently undamaged state of the embryos on first removal, that death was caused by toxic substances formed by anaerobic respiration in the endosperm. An attempt was made to circumvent this by removing the embryos and placing on nutrient agar, and also to remove toxic material with charcoal; these attempts, however, failed.

Effects of anaerobic germination on subsequent development.

1. *Devernalisation and reveralisation of spring rye.* In 1936 a few tests were made with spring rye at 1 and 4 weeks' exposure to nitrogen during germination at 20° C. Only those given one week's anaerobic treatment survived, and the plants were later grown to maturity. The results are given in Table IV.

TABLE IV

Spring Rye (Petkus) 1936. Retarding Effect on Flowering of Anaerobic Treatment at 20° C. during Germination.

Treatment.	Leaf number.	Days to anthesis.
Anaerobic 1 week . . .	8.0	64.0
Control	7.1	57.3

There appeared strong indication of a delay in flowering. This might be attributed to injury by the anaerobic conditions, but the increase in leaf number appeared significant as leaf number has always been found to characterize the stage of vernalization (Purvis, 1934) (Purvis and Gregory, 1937). No treatment during germination previously utilized had shown a leaf number greater than seven in spring rye. In 1937 the experiment was repeated on a more extensive scale, and the results appear in Table V.

TABLE V

Spring Rye (Petkus) 1937. Devernalisation by Anaerobic Germination at 20° C.

Preliminary period in nitrogen.	Number of leaves on main axis.	Days to anthesis.	No. of replicates.
Control (none). . . .	6.80 ± 0.20	50.3 ± 0.49	10
1 week	7.52 ± 0.11	52.6 ± 0.45	23
2 weeks	7.67 ± 0.13	54.4 ± 0.73	24
3 weeks	8.29 ± 0.36	57.4 ± 1.70	7

The earlier results are thus confirmed, and the standard errors given in the table clearly show that the results are highly significant both for leaf number and time to anthesis. The plants in this experiment are shown in Pl. XXX, Fig. 1.

The increase in leaf number suggests that by the anaerobic treatment spring rye has been converted into a condition approaching that of winter rye. Since in winter rye low temperature during germination reduces leaf number and hastens flowering, the crucial experimental was tried of chilling the spring rye seeds after they had already been exposed to anaerobic conditions. After varying periods of treatment in nitrogen at 20° C. the rye grain was then kept for three weeks at 1° C. previous to planting out. The results are presented in Table VI.

TABLE VI

Spring Rye (Petkus) 1937. Revernalisation after Anaerobic Treatment at 20° C. by Exposure to 1° C. in Air for 3 weeks.

Preliminary anaerobic treatment.	No. of leaves on main axis.	Days to anthesis.	No. of replicates.
Control (none).	6.80 ± 0.20	50.3 ± 0.49	10
1 week	—	47.3 ± 0.34	14
2 weeks	7.10 ± 0.23	50.5 ± 0.79	10
3 weeks	all died		

Comparable series in Tables V and VI show clearly that 'chilling' after anaerobic treatment again reduces leaf number to the normal for spring rye, and that the delay in flowering has been completely removed. These experiments show conclusively that the anaerobic treatment does not impair the later growth of such seeds as survive, and that therefore the effect is confined to processes occurring during germination. The devernalisation of spring rye by lack of oxygen and subsequent revernalisation by chilling are clearly established. This experiment lends considerable weight to the view that the effect of low temperature is specific.

2. *After-effect of anaerobic treatment on winter rye and wheat.* The surviving grain from the anaerobic treatments at 20° C. recovered their normal vigour and produced healthy plants which differed little from the controls. The values for spike development, tillering, and leaf number for winter rye and wheat are given in Tables VII and VIII.

TABLE VII

Winter Wheat (var. Lutescens 1060/10) 1936. Effect of Anaerobic Treatment during Germination on Development and Flowering.

Treatment.	Control.	Anaerobic at 1° C.		Air at 1° C.	Anaerobic at 20° C.
		12 weeks.	9 weeks.	9 weeks.	3 weeks.
Leaf number	23.2	23.7	25.4	7.7	24.4
Tilled number	11.7	8.6	10.1	7.8	17.0
Spike length mm.	0.3	0.6	0.3	ripe ears	0.3
'Score'	19	21	18	119	15

TABLE VIII

Winter Rye (Petkus) 1936. Effect of Anaerobic Treatment during Germination on Development and Flower Production

Treatment.	Control.	Anaerobic.		Air.
		9 weeks at 1° C.	1 week at 20° C.	9 weeks 1° C.
Leaf number . . .	23·3	23·4	22·3	8·1
Tilled number . . .	9·3	9·3	7·7	—
Spike length mm. . .	1·2	2·4	1·9	ripe ears
'Score'	21	25	21	133

The most striking result seen in Tables VII and VIII is the complete inhibition of the effect of low temperature on flower production in the absence of oxygen. This confirms the statement of Lysenko (I.A.B. Bull. 16, p. 8) that oxygen is required for vernalisation. The 'score' entered in the tables represents the stage of development reached in conventional units as described previously (Gregory and Purvis, 1938, p. 242). The leaf number of the plants germinated anaerobically reaches the maximum found in unvernalsed plants or those grown in continuous short days (Purvis and Gregory, 1937). The very small effect of prolonged anaerobic treatment at 1° C. on the development of the spike seen in both rye and wheat is the only indication of the after-effect of low temperature.

Effect of very low oxygen tension on vernalisation.

Since vernalisation by low temperature appeared to be inhibited completely by anaerobic conditions it was of interest to investigate the effect of very low oxygen tension. The experiment was performed in the following way. Imbibed grain was kept in a slow stream of nitrogen to which oxygen was added by allowing air to diffuse through capillaries of the requisite length and bore. The grain was placed in glass boiling tubes provided with a cork through which passed two tubes for passage of nitrogen and also a capillary tube connected with the air. To obviate gas flow through the capillary, due to pressure differences caused by temperature changes, each tube was placed in a thermos flask. The tubes were connected in parallel to a cylinder of compressed nitrogen, through a flow meter, pyrogallol towers, and finally a high capillary resistance so that the nitrogen entered the tubes at atmospheric pressure. The partial pressure of oxygen was maintained at $\frac{1}{500}$ normal. The results for winter rye are given in Table IX.

From the results in Table IX it is clear that a considerable degree of vernalisation has occurred with the low oxygen tension of $\frac{1}{500}$ normal, both from leaf number and spike-length data. The tension of oxygen required is thus very low.

Effect of varying concentration of carbon dioxide.

That high concentrations of CO₂ may inhibit germination is well known, and also that germination occurs after removal of the gas. Kidd (1914),

working with barley, found that 12 per cent. of CO₂ scarcely affected germination, but that at 35 per cent. CO₂ germination failed. On removing the gas even after exposure to 96 per cent. CO₂ some grain germinated.

In these experiments mixtures of air and CO₂ of varying concentration were prepared and passed over the imbibed grain. Up to 20 per cent. CO₂

TABLE IX

Winter Rye (Petkus). Effect of Partial Pressure of Oxygen ($\frac{1}{500}$ normal) during Germination on Germination and Development.

Treatment.	6 weeks.		3 weeks.	Unvernalised
	$\frac{O_2}{500}$ at 1° C.	Normal O ₂ at 1° C.	$\frac{O_2}{500}$ at 20° C.	3 days at 20° C. in air.
Percentage germination . . .	90.8	95.0	50.5	95.0
Leaf number	18.7	10.5	23.0	21.0
Spike length mm.	74.8	ripe ears	2.1	3.2
'Score' (29/9/37)	48	127	23	26

germination was as rapid as in air, but at higher levels germination fell off. In 30 per cent. CO₂ after three weeks at 20° C. 36 per cent. had germinated; at 50 per cent. CO₂, 1.5 per cent.; at 100 per cent. CO₂ complete inhibition was found. The effect of the carbon dioxide on the resulting plants in the case of winter rye was small, a slight retardation in spike development alone was found in comparison with unvernalsed grain germinated in air. In the case of spring rye treated for three weeks at 20° C. a strong retardation was noted in flowering and an increase in leaf number. This effect increased with rising concentration of CO₂ up to 20 per cent., above which level no further change was noted. These effects are seen from the data in Table X.

TABLE X

Spring Rye (Petkus). Effect of Varying Concentration of Carbon Dioxide during Germination on Flowering.

Percentage CO ₂ .	Air.	5.	10.	20.	30.	50.	100.
Leaf number	6.8	8.1	8.0	8.0	8.3	—	—
Days to anthesis	50.3	54.8	56.0	61.0	60.0	60.0	60.6

The increase in leaf number and delay in flowering suggest that high concentration of carbon dioxide leads also to devernalsation of spring rye.

The specific nature of the low-temperature effect.

Evidence of the specific nature of the low-temperature effect has been discussed in previous papers. It appeared desirable to test whether this effect of low temperature was reversible by high temperature. That a reversal of vernalisation is possible has been shown very clearly by drying down vernalised grain for varying periods both by the present authors and by Lojkin (1936) (Gregory and Purvis, 1937, p. 246).

The control of germination by anaerobic conditions made possible a direct investigation of the effects of subsequent periods of high temperature after previous exposure to low temperature. It may be stated at the outset that high temperatures do not annul the effect of chilling after germination has reached an advanced stage, since were this the case no after-effects of low temperature could appear.

The alternate exposures to low and high temperature must therefore be



TEXT-FIG. 2. Apparatus used for exposing imbibed grains alternately to nitrogen and air. For details see text.

given in the early stages of germination, and to make this possible some means of inhibition during the high-temperature periods is essential. The method used is briefly as follows.

The grain (5 gm. Petkus winter rye) previously sterilized was placed in horizontal tubes as shown in Text-fig. 2, and moistened with 3 c.c. of sterile tap-water. Three such tubes were used for each treatment. Each batch of seed received in all six weeks of low-temperature ($1^{\circ}\text{C}.$) treatment in air with periods at $20^{\circ}\text{C}.$ for twenty-four hours in pure nitrogen interspersed at varying intervals of time. The treatments are shown below in Table XI.

TABLE XI.

Treatments given in Experiments with Alternating High and Low Temperature

Treatment.	Total time at $1^{\circ}\text{C}.$ in air.	Total time at $20^{\circ}\text{C}.$ in nitrogen.	Time in N_2 at $1^{\circ}\text{C}.$ Time in air at $20^{\circ}\text{C}.$
1 day at $1^{\circ}\text{C}.$ followed by 1 day at $20^{\circ}\text{C}.$	6 weeks	6 weeks	1 : 1
2 days at $1^{\circ}\text{C}.$ " 1 "	6 weeks	3 weeks	1 : 2
3 " $1^{\circ}\text{C}.$ " 1 "	6 weeks	2 weeks	1 : 3
6 " $1^{\circ}\text{C}.$ " 1 "	6 weeks	1 week	1 : 6

During the warm periods purified nitrogen was passed through the tubes connected in series by rubber tubes. In course of transfer from cold to warm conditions the tubes were kept in a bath of ice and nitrogen passed to sweep out air. The temperature of the bath was then raised rapidly to $20^{\circ}\text{C}.$ and kept so for twenty-four hours. In the reverse transfer from warm to cold conditions the tubes were closed by clipping the rubber connexions and placed in the refrigerator for one hour, after which time the clips were removed and the nitrogen drawn out of the tubes, which were left in contact

with air. In this way the seeds were never exposed to high temperature in the presence of oxygen, so that at the end of periods of treatment the grains alternately kept at 1° C. and 20° C. had germinated no farther than controls kept continuously for six weeks in air at 1° C. The grain during these treatments was kept always in the dark.

Three separate control series were used: (1) unvernalsed seed germinated at 20° C. in air; (2) seed kept continuously at 1° C. for twelve weeks in the refrigerator and exposed for alternate twenty-four-hour periods to air and pure nitrogen; (3) seed vernalised in the usual way at 1° C. in air for six weeks. The treatments were so arranged that all ended at the same time and the seed was planted out on the same day.

The effects of these treatments on germination showed that where the total period of anaerobic conditions at 20° C. was six weeks very considerable reduction resulted, but less than with an equal period of continuous anaerobic treatment (cf. Table III). These results, and the data for leaf number and flower production are given in Table XII.

TABLE XII

Winter Rye (Petkus). The Effect of Alternate Exposures to Low Temperature in Air and High Temperature in Nitrogen on Germination, Leaf Number, and Flower Production

	Controls.			Ratio $\frac{\text{total time at 20° C. in N}_2}{\text{total time at 1° C. in air.}}$			
	1.	2.	3.	1 : 1	1 : 2	1 : 3	1 : 6
Final leaf number . . .	23.0	8.9	10.5	24.0	—	—	11.0
Days to first anthesis . . .	∞	55	70	∞	106	87	69
'Score' (16 Sept.) . . .	21	129	127	21	44	70	108
Percentage germination . . .	76	55	—	8	43	46	50

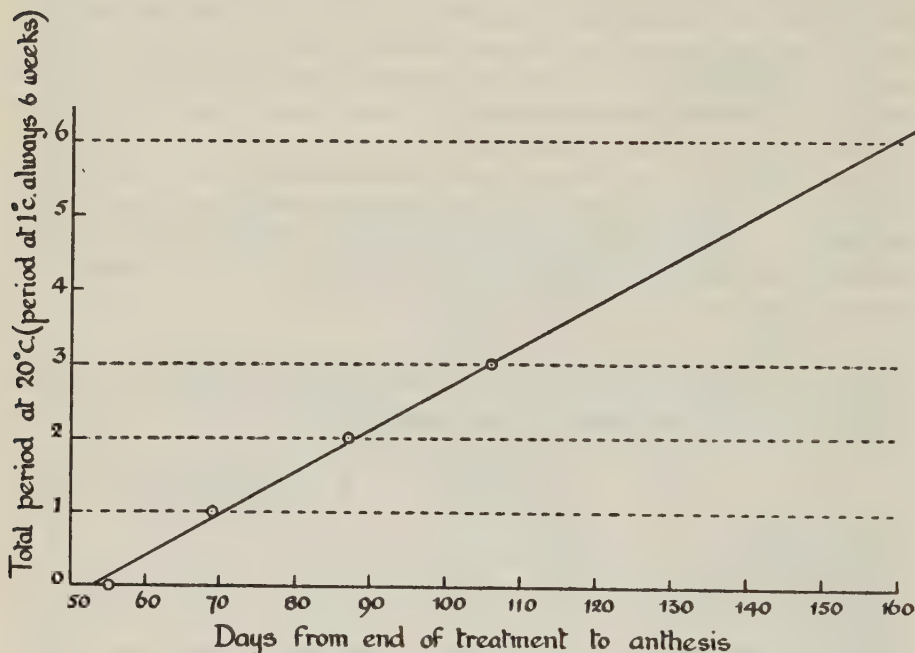
Controls (1) unvernalsed, (2) 12 weeks at 1° C. alternate days in air and nitrogen, (3) vernalised 6 weeks in air at 1° C.

In Text-fig. 3 is shown the relation for each series between time to first anthesis (i.e. until at least one plant of series has exserted anthers) and the total duration of exposure to high temperature. The relation is strictly linear. At the time of examination of the plants those in the series with daily alternatives (ratio 1 : 1) had not yet reached anthesis. By extrapolation from the curve the time should have been approximately 160 days. As recorded in a previous paper (Purvis and Gregory, 1937, p. 572) the time taken for unvernalsed plants to come into ear is about 170 days, which agrees well with the extrapolated value. Regarding now the data presented in Table XII the following points appear:

1. Twelve weeks at 1° C., of which six were under anaerobic conditions and six in air, have an effect similar to six weeks of vernalisation.
2. Six weeks total exposure to 1° C., but alternating daily with periods at 20° C. in nitrogen, leads to plants indistinguishable from unvernalsed.
3. Intermediate treatments with relatively longer exposures in air at 1° C. give intermediate results, showing that the delay in flower production

is strictly proportional to the total time at high temperature. (Total duration at 1°C . is in all cases six weeks.)

Comparison of the two control series 2 and 3 shows that the extra six weeks in series 2 in which the grain was kept at 1°C . in nitrogen has not resulted in an increase in time to anthesis. Anaerobic conditions therefore, as such, do not lead to a reversal of the vernalising process; indeed, in series 2 the



TEXT-FIG. 3. Relation between time to anthesis and the total period at 20°C . interspersed as 24-hour periods at regular intervals of 1, 2, 3, and 6 days in a total period at 1°C . of six weeks' duration.

time to anthesis is somewhat reduced below that of the vernalised series 3. Possibly the oxygen present in the grain at the time of transference to nitrogen is sufficient to allow the low-temperature process to continue for some time, so that the extra six weeks given in series 2 may be equivalent to a larger vernalising period. This need not be further discussed.

The important fact which emerges is that no reversal appears at low temperature in nitrogen. Comparing control series 2 with the series receiving alternating daily exposures to air and nitrogen at 1°C . and 20°C . respectively establishes without doubt that it is the high temperature and not the anaerobic conditions that is mainly concerned with the reversal of vernalisation. The plants in this experiment are shown in Pl. XXX, Fig. 2.

It does not appear necessary to discuss again the theoretical considerations raised by the experiments here reported, as the views held by the authors have already been put forward at some length in a previous communication

(Purvis and Gregory, 1937, p. 583 et seq.). The results here described were referred to in the paper cited (p. 587) and the evidence for the statements there made has now been presented.

In conclusion the authors wish to record their indebtedness to Mr. R. V. Martin, technical assistant of this Institute, for his help in construction of the apparatus and assistance in carrying through a complicated schedule without a hitch.

SUMMARY

Two effects of low temperature during germination are considered, namely, mere delay in germination and the specific vernalising effect. These were experimentally separated by using anaerobic conditions and high concentrations of carbon dioxide as a means of prolonging germination at high temperature.

Plants of winter rye and wheat, and of spring rye were found to grow to maturity after a maximum exposure of three weeks in nitrogen at 20° C. and twelve weeks at 1° C. Anaerobic conditions resulted in 'devernalisation' of spring rye with increase in leaf number and delay in anthesis (Table V). A similar effect was noted with carbon-dioxide concentrations above 20 per cent. (Table X). Spring rye previously devernalised by anaerobic conditions could again be reveralised by low temperature giving them a normal leaf number and a normal time to anthesis (Table VI).

Anaerobic conditions completely prevent vernalisation even after twelve weeks at 1° C. (Table VII).

The tension of oxygen required for complete vernalisation is greater than $\frac{1}{500}$ normal, though at this level considerable vernalisation occurs (Table IX).

The specific effect of low temperature was proved by exposing imbibed grain alternately to air at 1° C. and nitrogen at 20° C. Equal daily exposures for twelve weeks gave plants indistinguishable from unveralised, while controls with similar alternations of air and nitrogen kept at 1° C. were completely vernalised.

Alternations of six days at 1° C. in air followed by one day at 20° C. in nitrogen (total time in air at 1° C. six weeks) gave completely vernalised plants. Using the same total period of low temperature, intermediate values were obtained with different relative durations at the two temperatures, and a linear relation is found between time to anthesis and total duration at 20° C. High temperatures bring about quantitative reversal of the low-temperature effect and the specific nature of the action of low temperature is proved.

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EXPLANATION OF PLATE XXX

Illustrating Prof. F. G. Gregory's and Dr. O. N. Purvis's paper on 'Studies in the Vernalisation of Cereals. III. The Use of Anaerobic Conditions in the Analysis of the Vernalising Effect of Low Temperature during Germination.

Fig. 1. Devernalisation of spring rye by anaerobic treatment at 20° C. during germination. The duration of treatment in weeks is given. The delay in flowering due to the anaerobic treatment is evident. The ears in the control series are past anthesis while those in series 3 have just reached that stage. Photograph taken eight weeks from planting.

Fig. 2. Reversal of vernalisation by periods of high temperature. The numerals show the ratio of the time of exposure at 1° C. to the time at 20° C., the total duration of chilling being six weeks in each case. The control series were kept for twelve weeks at 1° C., with daily alternations in air and in nitrogen. Series 1/1 completely unvernalsed, 6/1 completely vernalised. (Cf. Text-fig. 3.) Photograph taken ten weeks from planting.



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Huth, Stubbs X Kent.

GREGORY & PURVIS - VERNALISATION

**The Interaction of Light Intensity and Nitrogen Supply in
the Growth and Metabolism of Grasses and Clover
(*Trifolium repens*)**

**II. The Influence of Light Intensity and Nitrogen Supply on the Leaf
Production of Frequently Defoliated Plants**

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With thirteen Figures in the Text

INTRODUCTION

THE first paper of this series (Blackman, 1938) contained an account of field experiments in which the effects of varying both light intensity and nitrogen supply on the clover (*Trifolium repens*) content of a sward were investigated. The results demonstrated that there was a marked interrelationship between the light and nitrogen effects. In daylight, the addition of calcium nitrate, and more particularly ammonium sulphate, diminished considerably the amount of clover. Similarly, decreasing the light intensity to 0.6 or 0.4 of daylight also brought about a large reduction of the clover, but additional nitrogen at these lower light levels did not reduce the content further. These findings showed that at the highest light intensity the greater reduction of the clover caused by ammonium sulphate as against calcium nitrate could not be ascribed to a toxic accumulation of ammonium ions within the plant. There was alternative evidence that the depression of the clover was associated with the active growth of the grasses. It seemed probable that the failure of additional nitrogen to diminish the clover under reduced light intensity was due to differences in the reaction of grasses and clover to nitrogen at the lower light levels.

In the present series of experiments the effects of light intensity and nitrogen supply on the growth of *Agrostis tenuis*, *Festuca rubra*, and *T. repens* have been studied. Since in the previous investigation the sward was cut at short intervals, in the present series of experiments the plants were frequently

defoliated. For the same reason, the levels of light intensity employed and quantities of nitrogen added were similar to those of the field experiments already described (Blackman, 1938).

EXPERIMENTAL RESULTS

Experimental technique.

All the present experiments were carried out either on plants grown in pots or on swards previously established on specially levelled areas. In the 1933 experiments the pots were filled with a mixture of sand, bentonite, calcium carbonate, and superphosphate in the proportions 94.7 : 2.5 : 2.5 : 0.3, while the other essential elements (N, K, Mg, S, Fe) were added in solution as inorganic salts. Subsequent to 1933, the potting mixture consisted of loam, sand, calcium carbonate, and superphosphate in the proportions of 73.7 : 25.0 : 1.0 : 0.3, and no additional nutrients except nitrogen were supplied in solution.

Clay pots (14 in. diam. $\times$ 8.5 in. deep) coated with paraffin wax were employed in 1933, while in the years 1934–6 glazed earthenware pots (10 in. diam. $\times$ 10 in. deep) were used. The large pots of the earlier experiment were so heavy as to make frequent moving impossible so they were watered when it was thought the occasion demanded. In 1934–6 the water content of the pots was maintained at 40–50 per cent. of the water-holding capacity. At the beginning of each experiment and at subsequent defoliations the herbage in the pots was cut back to within half an inch of the soil level by means of sheep shears, while in the field experiments a lawn mower was used to harvest the plots. Within an hour of cutting, the herbage was placed in an oven specially designed to ensure rapid drying.

The same practice as in the parallel investigation already described (Blackman, 1938) was employed to reduce the light intensity. The pots or plots were permanently shaded with butter muslin stretched one or more layers thick on light wooden frames. In the field the screens were fixed in position some 3 in. above ground-level, but in the pot experiments the distance between the screen and the top of the pot was 6 to 8 in. In 1933 the amount of light absorbed by the butter muslin was estimated by means of a 'Holophane' lumeter, samples of the material being tested from time to time in the laboratory. Subsequent estimations were carried out *in situ* by means of a 'Weston' phototronic cell coupled to a suitable milliammeter. During the period of each experiment, light intensity observations were carried out at frequent intervals. In the pot experiments each pot was in the nature of a 'sub-plot'; replicates both for species and manurial treatments were grouped together and constituted a plot. For example, in the 1935 pot experiment there were three species and three manurial treatments. Sets of nine pots, therefore, made up a plot, and these sets received the appropriate light treatment. In the 1934–6 experiments the arrangement of the pots in each 'plot'

was re-randomized several times during the experimental period. In the field experiments the replication was fourfold, and in the pot experiments seven- or eightfold.

The interaction of light intensity and nitrogen supply in leaf production.

1933 experiments.

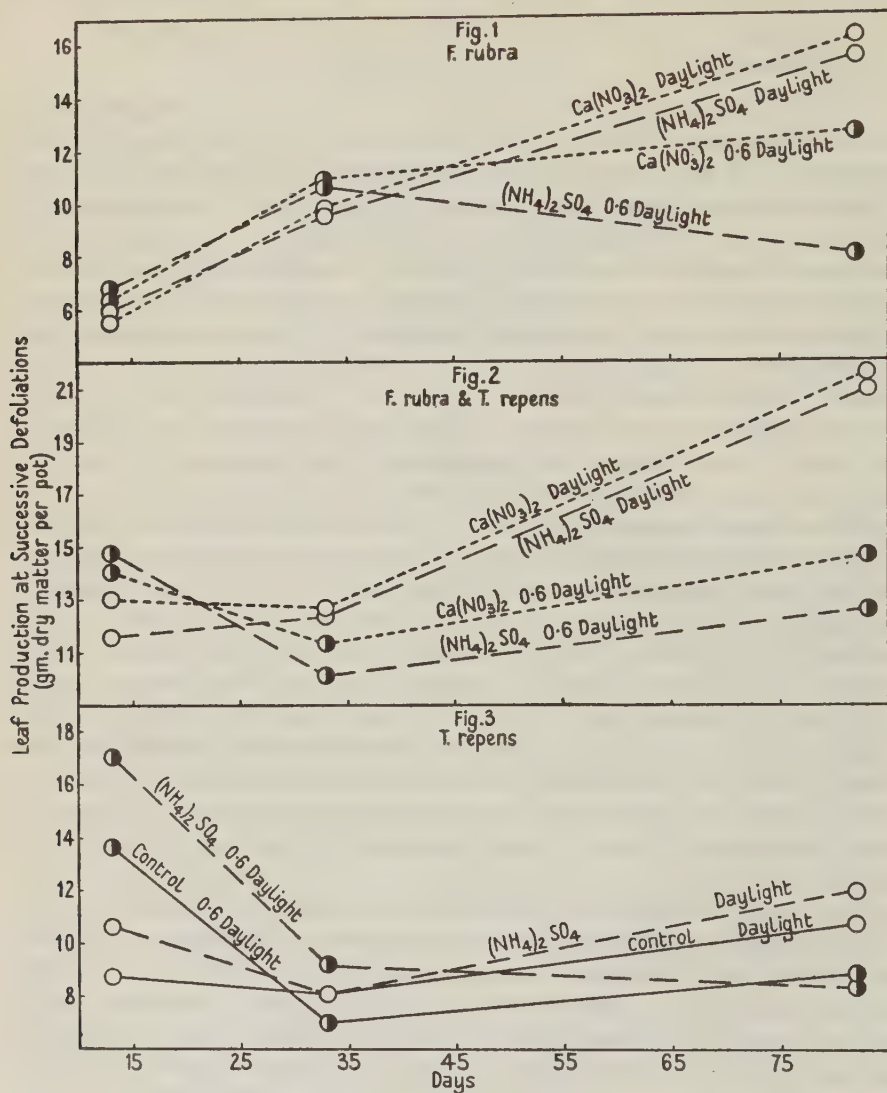
In this year three separate experiments were carried out. Seed of *F. rubra* and *T. repens* was sown either alone at the rates of 4.37 and 1.0 gm. per pot respectively, or as a mixture of 2.0 and 1.0 gm. Germination during April was satisfactory, although there was marked tendency for the sand-bentonite mixture to form a hard surface cake. The seedlings were allowed to grow freely until August 1, when all the pots were cut and the various experiments started. During the preliminary period each pot received 2.4 gm. potassium sulphate and 0.75 gm. magnesium sulphate. The pots which contained *F. rubra*, either alone or mixed with clover, were also given 3.0 gm. calcium nitrate, while the clover pots received 1.9 gm. These salts were applied as separate solutions, and in the case of calcium nitrate some ferric chloride was included. Three weeks after the experiments began, 0.25 gm. ferric chloride and 0.5 gm. potassium sulphate were added to each pot.

The two experiments carried out on *F. rubra* or a mixture of *F. rubra* and *T. repens* were of a similar nature. There were four treatments in all, two levels of light intensity and two manurial treatments. The lower light intensity was obtained by shading the pots with a single layer of butter muslin, which it was estimated reduced the light intensity to 0.6 of daylight. The pots received weekly either 4.8 gm. of calcium nitrate or the equivalent amount of nitrogen as ammonium sulphate. In the clover experiment the light intensities were the same, but calcium nitrate was omitted; thus half the pots received no additional nitrogen. In each experiment the herbage, which consisted almost entirely of leaves, was cut and collected on August 13, September 5, and October 1. The results obtained are shown in Figs. 1, 2, and 3, and Table I.

Considering first Figs. 1 and 2, it is seen that the trend of changes is very similar in both experiments. For the two earlier defoliations there is little difference between the several treatments. In the last cut, however, while in normal daylight both calcium nitrate and ammonium sulphate have similar effects, under the reduced light intensity with calcium nitrate added leaf production is much greater. Statistical analysis of the total yield for the three cuts (see Table I) shows that the interaction between the form of nitrogen and light intensity is significant in the case of *F. rubra*, and just not significant when *F. rubra* and *T. repens* are grown together. Furthermore, a reduction of the light intensity has depressed growth in both experiments.

In the clover experiment (see Fig. 3) the effects of reduced light intensity were somewhat different from those of the other two. In the first cut a reduction of the light intensity largely increased growth, and in the third cut

decreased it, but to a smaller extent. As a result the average effect over the experimental period was to increase significantly leaf production. At both light intensities the addition of ammonium sulphate had no significant



FIGS. 1-3. The effects of light intensity and nitrogen supply on the leaf production, when frequently defoliated, of *Festuca rubra* (Fig. 1), *Trifolium repens* (Fig. 3) and *F. rubra* and *T. repens* combined (Fig. 2). (Aug. 1-Oct. 23-4, 1933.)

influence on the total production of leaves. Nevertheless, at the lower light intensity the addition of ammonium sulphate tended at first to accelerate leaf production and subsequently to retard it; this trend is statistically significant.

TABLE I

The Interaction of Light Intensity and Nitrogen Supply in the
Leaf Production of *F. rubra* and *T. repens*

		Total leaf production (Aug. 1–Oct. 23, 1933) (gm. dry matter per pot).		
		<i>F. rubra</i> and <i>T. repens.</i>		
Treatments.		<i>F. rubra.</i>	<i>T. repens.</i>	<i>T. repens.</i>
1.0 Daylight	Control	—	—	27.5
	(NH ₄) ₂ SO ₄	43.61	43.21	30.54
	Ca(NO ₃) ₂	44.73	45.48	—
	Mean	44.18	44.35	29.02
0.6 Daylight	Control	—	—	33.67
	(NH ₄) ₂ SO ₄	32.58	35.57	35.55
	Ca(NO ₃) ₂	41.23	39.88	—
	Mean	36.91	37.73	34.61
Significant differences:				
(P = 0.05) (i) between treatments		7.55	4.56	6.08
(ii) between means of two treatments		5.34	3.23	3.59

1934 experiments.

These experiments were essentially of the same design as those of the previous year, save that the pots were smaller and the sand-plus-bentonite medium was replaced by the soil-sand mixture. During April seed of *F. rubra* and *T. repens* was sown singly or together at the rates 2.2 and 0.5 gm. per pot. The experiments were not started until July 30, when the herbage was first cut. Prior to this, the pots remained uncut, unshaded, and unmanured. For the experimental period there were three manurial treatments: (i) no nitrogen, (ii) calcium nitrate applied weekly at the rate of 2.16 gm. per pot, (iii) ammonium sulphate at a rate equivalent in nitrogen to the calcium nitrate. In addition, there were two light intensities, daylight and 0.62 of daylight (single layer of butter muslin). The pots were harvested three times, i.e. on August 13, September 5, and October 1.

It is not proposed to consider the results of these experiments in any detail, since unfortunately the data are not trustworthy. Subsequent to the first cut it was observed (i) that *F. rubra* was being attacked by a fungus of the *Macrosporium alternaria* type, and (ii) that the pots most affected were those which were both shaded and received additional nitrogen. Spraying with a Bordeaux malachite green mixture (Bennett, 1933) was not very effective, and the experiments were eventually abandoned. Simultaneously it was also found that although the soil, as a precaution, had been taken from a field in which no leguminous crop had been grown for many years, stem-eelworms (*Tylenchus dipsacae*) were present in the tissue of the normally resistant *T. repens*.

TABLE II

*The Interaction of Light Intensity and Nitrogen Supply in the
Leaf Production of F. rubra and T. repens*

					Total production of leaves (July 30–October, 1934) (gm. dry matter per pot).		
					<i>F. rubra</i> and <i>T. repens.</i>		
Treatments.					<i>F. rubra.</i>	<i>T. repens.</i>	<i>T. repens.</i>
1.0 Daylight	Control	.	.	.	5.43	17.23	18.67
	(NH ₄) ₂ SO ₄	.	.	.	31.84	32.38	22.97
	Ca(NO ₃) ₂	.	.	.	29.72	27.37	19.68
0.62 Daylight	Control	.	.	.	6.49	13.06	13.19
	(NH ₄) ₂ SO ₄	.	.	.	23.13	19.93	11.15
	Ca(NO ₃) ₂	.	.	.	17.60	16.52	10.01

In spite, however, of the insect and fungal attack, the data are worthy of some consideration in view of the results obtained in later experiments. The total leaf production for the three cuts is set out in Table II. In the case of *F. rubra*, additional nitrogen at both light levels largely increased growth. Lowering the light intensity did not depress the leaf production in the control, but where nitrogen was added regeneration was depressed, more particularly in the case of calcium nitrate. The mixture of *F. rubra* and *T. repens* gave very similar results, except that diminishing the light intensity produced similar effects with both forms of nitrogen. Clover grown alone, on the other hand, reacted very differently to the various treatments. In the first place, neither ammonium sulphate nor calcium nitrate increased leaf production; in the second place, the clover was more sensitive than the grass to a reduction in the light level.

1935 experiments.

Pot experiments. During 1935 both pot and field experiments were undertaken. In the pot experiments seed of *A. tenuis*, *F. rubra*, and *T. repens* at the rates of 1.7, 2.2, and 0.5 gm. per pot was sown during April. The experiments, compared to those of 1933–4, were started after a shorter preliminary period, namely, on June 18. Each of the three ‘balanced’ experiments contained nine treatments. There were three light intensities: (i) daylight, (ii) 0.61 daylight (single layer of butter muslin), (iii) 0.44 daylight (double layer of muslin). The manurial treatments were (i) control, (ii) ammonium sulphate at the rate of 1.0 gm. per pot every ten days, (iii) calcium nitrate at a rate of nitrogen equivalent to (ii). The pots in each experiment were cut back six times; the first four cuts were made at ten-day intervals, the last two after fourteen-day periods.

The results for the *F. rubra* experiment are shown in Fig. 4 and Tables III and IV. It is seen from Fig. 4 that the differences between the effects

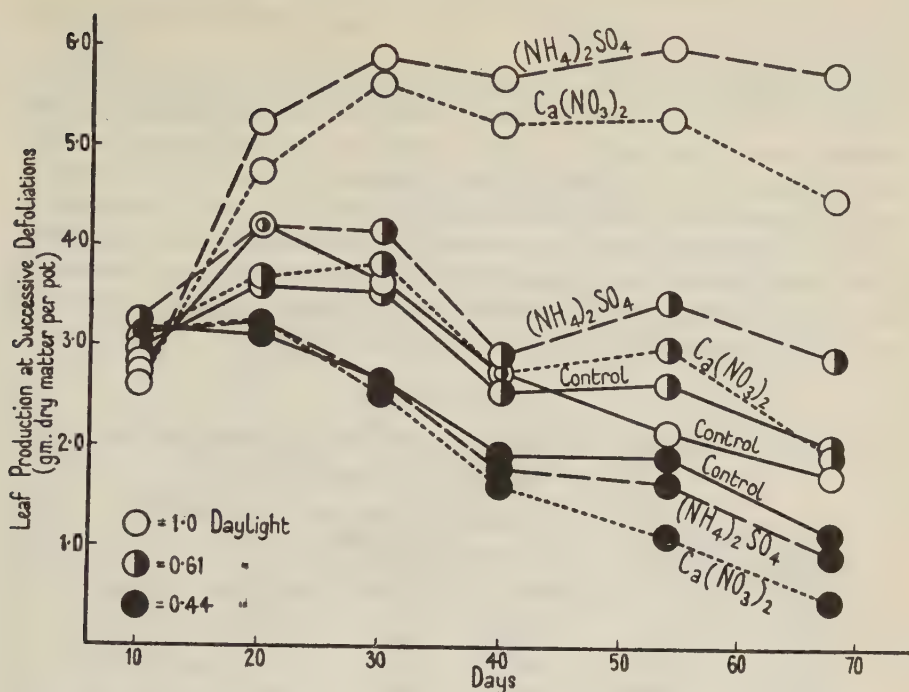


FIG. 4. The effects of light intensity and nitrogen supply on the leaf production of *Festuca rubra* when frequently defoliated. (June 18–Aug. 26, 1935.)

TABLE III

The Interaction of Light Intensity and Nitrogen Supply in the Leaf Production of F. rubra

Light treatments.	Total production of leaves (June 18–August 26, 1935) (gm. dry matter per pot).			
	Nitrogen treatments.			Mean.
Daylight.	Control.	(NH ₄) ₂ SO ₄ .	Ca(NO ₃) ₂ .	
1.0 . . .	17.19	31.51	28.16	25.62
0.61 . . .	17.37	20.94	18.27	18.86
0.44 . . .	13.92	13.28	12.14	13.11
Mean	16.16	21.91	19.52	

Significant difference (i) between treatments = 2.35
($P = 0.05$) (ii) between means of three treatments = 1.36

of the various treatments on leaf regeneration become progressively greater with successive cuts. The total leaf production for the six cuts is markedly affected by both the light intensity and nitrogen supply (see Table III). A reduction in the light level to 0.61 depresses growth with either calcium nitrate or ammonium sulphate added, but does not do so in the control. A

further diminution in the light intensity to 0.44 diminishes leaf production with all manurial treatments.

In full daylight, ammonium sulphate increases growth more than calcium nitrate. At the 0.61 level the ammonium sulphate effect is again significantly different from that of calcium nitrate. While at this light intensity calcium nitrate does not increase growth, there is evidence that with 0.44 daylight it actually depressed leaf production in the later cuts. In Table IV the statistical analysis of the data for the fifth and sixth cuts summed together shows that at this stage the depression is significant. In addition, it is seen that during this period the lower the light intensity the greater is the difference between the effects of ammonium sulphate and calcium nitrate.

TABLE IV

The Interaction of Light Intensity and Nitrogen Supply in the Leaf Production of F. rubra

Light treatments.	Production of leaves (cuts 5 and 6) (gm. dry matter per pot).			
	Nitrogen treatments.			Mean.
Daylight.	Control.	(NH ₄) ₂ SO ₄ .	Ca(NO ₃) ₂ .	
1.0	3.91	11.84	9.88	8.54
0.61	4.70	6.42	4.98	5.37
0.44	3.04	2.59	1.59	2.41
Mean	3.88	6.95	5.48	
Significant difference (i) between treatments				= 0.77
(P = 0.05) (ii) between means of three treatments				= 0.56

The effects of varying light intensity and nitrogen supply on the growth of *A. tenuis* are very similar to those for *F. rubra*. While the initial differences between the several treatments are small, they are very marked in the later cuts (see Fig. 5). The statistical analysis of leaf production data for the whole experimental period (see Table V) shows that a decrease in the light intensity first to 0.61 and then to 0.44 in every case depresses growth. In daylight, calcium nitrate, and more particularly ammonium sulphate, largely increases leaf production. At the level of 0.61, calcium nitrate in contrast to ammonium sulphate causes a reduction in yield, while at the lowest light intensity both forms of nitrogen depress leaf production.

The behaviour of *T. repens* differs in many respects from that of either *F. rubra* or *A. tenuis*. There is a similar trend in regard to the light treatments, but not in regard to nitrogenous manuring (see Fig. 6 and Table VI). Whatever the nitrogen treatment, a reduction in the light level first to 0.61 and then to 0.44 suppresses growth. At all three light intensities, ammonium sulphate, but not calcium nitrate, increases leaf production by a small amount. At the lowest and highest intensity the difference between ammonium sulphate and calcium nitrate is significant. In contrast with the results obtained for the two grasses, a reduction of the light intensity has not caused marked differences in the effects of ammonium sulphate and calcium nitrate.

Field experiments. The two field experiments were laid out on two adjacent swards which had been specially prepared. In the previous autumn the areas had been levelled and seed of either *A. tenuis* or *T. repens* sown. During the

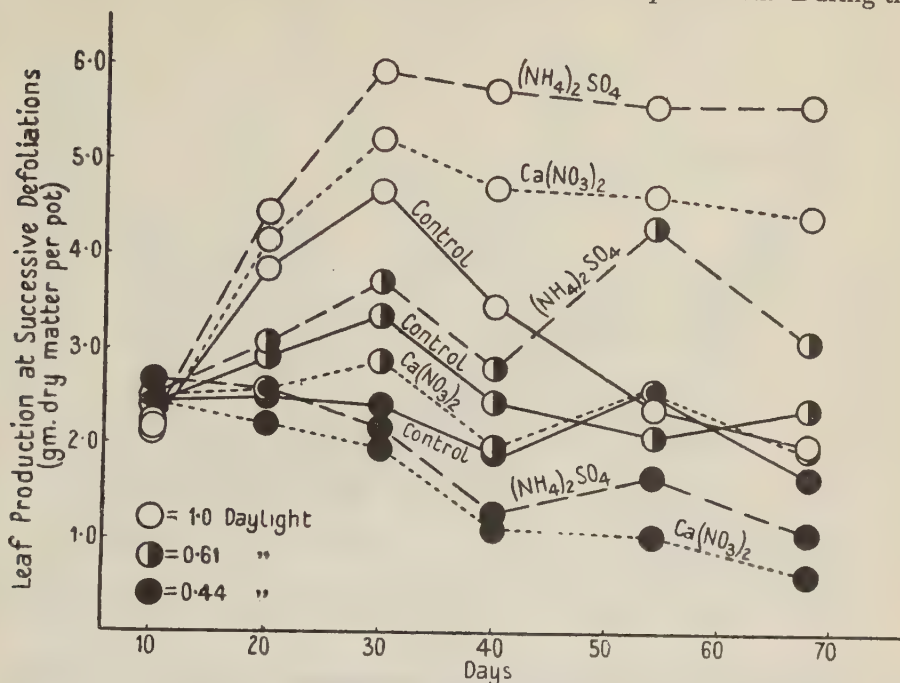


FIG. 5. The effects of light intensity and nitrogen supply on the leaf production of *Agrostis tenuis* when frequently defoliated. (June 18-Aug. 26, 1935.)

TABLE V

The Interaction of Light Intensity and Nitrogen Supply in the Leaf Production of A. tenuis

			Total production of leaves (July 18–August 26, 1935) (gm. dry matter per pot).			
Light treatments.			Nitrogen treatments.			
Daylight.			Control.	(NH ₄) ₂ SO ₄ .	Ca(NO ₃) ₂ .	Mean.
1.0	.	.	18.44	29.90	25.23	24.52
0.61	.	.	16.58	19.45	14.42	16.82
0.44	.	.	13.49	11.31	9.35	11.38
Mean			16.17	20.22	16.33	
Significant difference (i) between treatments						= 1.51
(P = 0.05) (ii) between means of three treatments						= 0.87

preliminary period of establishment the turf was frequently weeded by hand and kept short by occasional cutting. The two experiments were very similar in design to the pot experiments. There were three levels of daylight (full,

0.61 and 0.44 of daylight), and three manurial treatments. The ammonium sulphate and calcium nitrate were added weekly at a rate some 50 per cent.

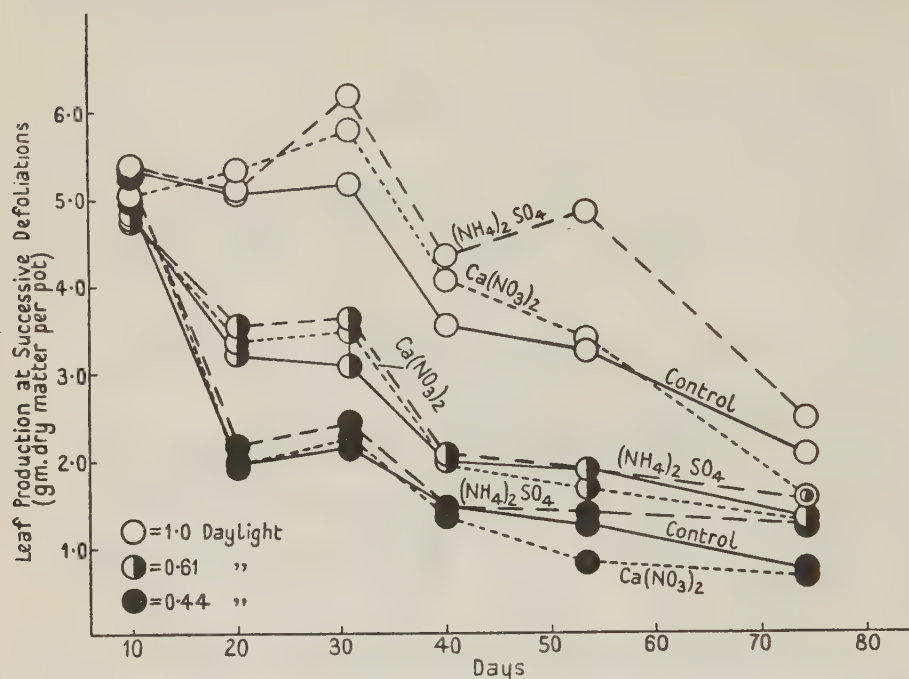


FIG. 6. The effects of light intensity and nitrogen supply on the leaf production of *Trifolium repens* when frequently defoliated. (June 18–Sept. 2, 1935.)

TABLE VI

The Interaction of Light Intensity and Nitrogen Supply in the Leaf Production of T. repens

		Total production of leaves (June 18–September 2, 1935) (gm. dry matter per pot).			
Light treatments.		Nitrogen treatments.			Mean.
Daylight.	Control.	(NH ₄) ₂ SO ₄ .	Ca(NO ₃) ₂ .		
1.0	.	24.32	27.24	25.16	25.57
0.61	.	15.80	17.35	16.42	15.52
0.44	.	12.51	13.96	12.27	12.91
	Mean	17.54	19.52	17.95	
Significant difference (i) between treatments					= 1.29
(P = 0.05) (ii) between means of three treatments					= 0.75

greater than in the pots, i.e. 4.6 gm. nitrogen per square yard. It was planned to cut the plots every seven days, but after the middle of September the high rainfall and the consequent sodden surface made the intervals irregular.

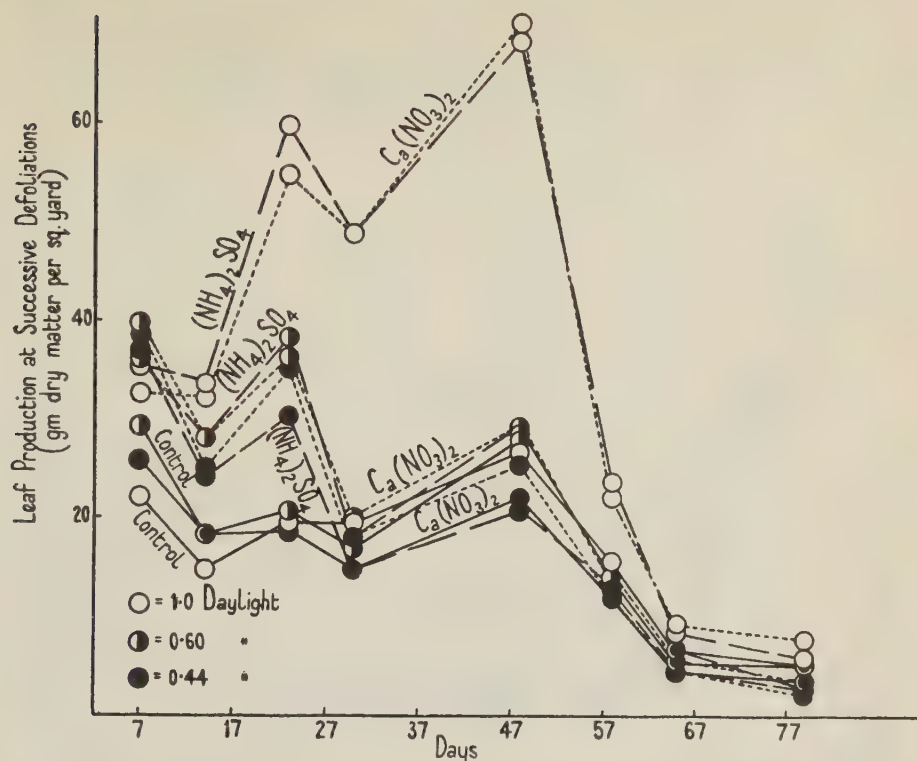


FIG. 7. The effects of light intensity and nitrogen supply on the leaf production of *Agrostis tenuis* when frequently defoliated. (Aug. 14–Nov. 1, 1935.)

TABLE VII

The Interaction of Light Intensity and Nitrogen Supply in the Leaf Production of A. tenuis

		Total production of leaves (August 14–November 1, 1935) (gm. dry matter per sq. yard).			
Light treatments.	Daylight.	Nitrogen treatments.			Mean.
		Control.	(NH ₄) ₂ SO ₄ .	Ca(NO ₃) ₂ .	
1.0	.	130.50	283.95	277.94	230.80
0.61	.	137.59	172.91	179.54	163.34
0.44	.	120.53	147.63	162.40	143.51
	Mean	129.54	201.50	206.63	
Significant difference (i) between treatments		= 24.50			
(P = 0.05)		(ii) between means of three treatments = 14.15			

The results obtained for *A. tenuis* are set out in Fig. 7 and Table VII. During the course of the experiment there was a gradual fall in temperature from August to November, and it seems clear from Fig. 7 that after the fifth

cut, temperature rather than light intensity or nitrogen supply was the principal factor controlling growth. Where no nitrogen was added the production of leaves was not significantly affected by diminutions in the light intensity. At all three light levels both ammonium sulphate and calcium

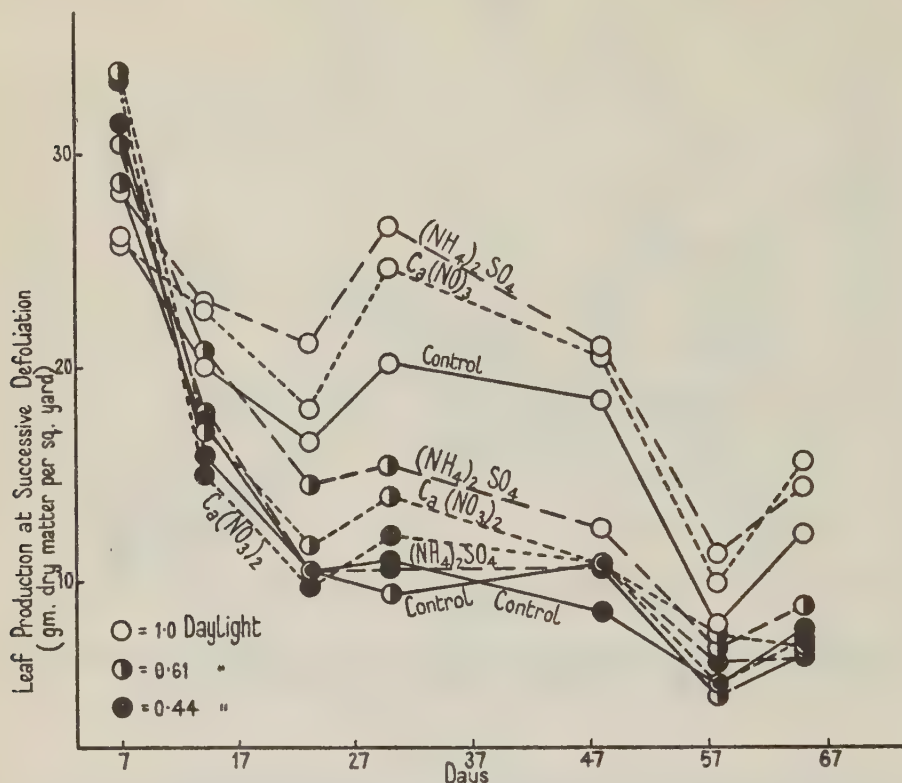


FIG. 8. The effects of light intensity and nitrogen supply on the leaf production of *Trifolium repens* when frequently defoliated. (Aug. 14–Oct. 18, 1935.)

nitrate increased growth, but the magnitude of the increase was by far the greatest in full daylight. Unlike the results obtained in the pot experiment, there was no correlation between light intensity and differences in the effects of both ammonium sulphate and calcium nitrate.

In the clover experiment, the seasonal fall in temperature is again reflected in the downward trend of leaf production with successive cuts (see Fig. 8). Over the whole period of the experiment, the addition of either ammonium sulphate or calcium nitrate increased growth significantly at all but the lowest light intensity (see Table VIII). Where nitrogen was added, a reduction in the light level, first to 0.61 daylight and then to 0.44, in general diminished leaf production. In the absence of additional nitrogen, however, the reduction from 0.61 to 0.44 daylight caused no significant diminution.

TABLE VIII

The Interaction of Light Intensity and Nitrogen Supply in the Leaf Production of T. repens

Total production of leaves (August 14–October 18, 1935) (gm. dry matter per sq. yard).					
Light treatments.		Nitrogen treatments.			
Daylight.	Control.	(NH ₄) ₂ SO ₄ .	Ca(NO ₃) ₂ .	Mean.	
1.0	118.64	141.74	135.34	131.90	
0.61	94.68	105.98	98.93	96.53	
0.44	87.32	90.47	88.04	88.61	
Mean	96.88	112.73	107.43		
Significant difference (i) between treatments				= 8.80	
(P = 0.05) (ii) between means of three treatments				= 5.08	

1936 experiments.

The experimental procedure in this year was somewhat different from the previous pot experiments. Instead of seed being sown in the pots, circular turves (10 in. in diameter and 5 in. thick) were fitted into the pots (10 in. diameter), the remainder of the pot being filled with the standard potting mixture (see p. 766). These turves of either *A. tenuis* or *T. repens* were obtained from the two swards on which the field experiments of 1935 were carried out. The experiments were begun some six weeks after the transference to the pots, i.e. on May 8. As in the pot experiments of 1935, there were three levels of light intensity (i) daylight, (ii) 0.63 daylight, and (iii) 0.37 daylight (i.e. triple layer of butter muslin). The three manurial treatments consisted of (i) control, (ii) ammonium sulphate at the rate of 1.0 gm. per pot every ten days, (iii) calcium nitrate at an equivalent nitrogen rate. The pots were cut six times at ten-day intervals.

The results for *A. tenuis* are set out in Fig. 9 on a somewhat different basis from previous figures. For the purpose of clarity the production of leaf tissue for each treatment is expressed relatively to the control (1.0 daylight with no nitrogen), which at each cut has been taken as equal to 100. It is seen that the results show trends very similar to those of the pot experiment of 1935. The figures for leaf production over the whole experimental period (see Table IX) show that ammonium sulphate and calcium nitrate have increased growth equally in full daylight. On the other hand, at the two lower light levels the effect of ammonium sulphate is significantly greater than that of calcium nitrate. A reduction in the light intensity from daylight to 0.63 daylight has not affected leaf regeneration in the absence of additional nitrogen, but has led to a decrease where ammonium sulphate, or more particularly calcium nitrate, was applied. A further decrease in intensity from 0.63 to 0.37 caused similar effects.

It would appear from Table IX that at the lowest light intensity the addition of neither calcium nitrate nor ammonium sulphate caused a significant change

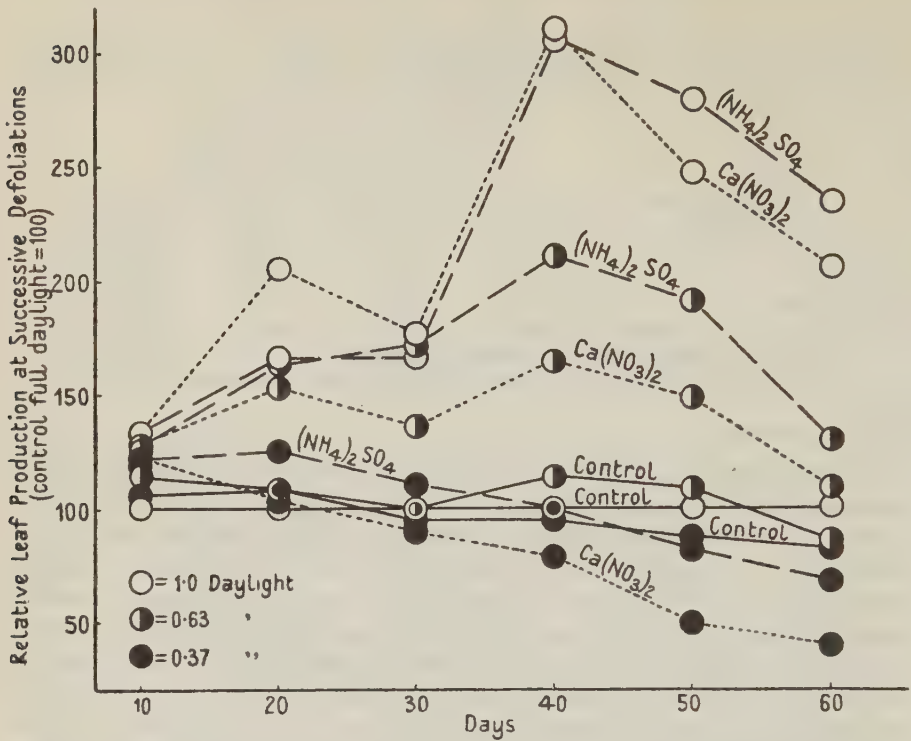


FIG. 9. The effects of light intensity and nitrogen supply on the leaf production of *Agrostis tenuis* when frequently defoliated. At each defoliation the leaf production for the individual treatments has been expressed relatively to the amount produced on the control receiving full daylight. (May 8–July 9, 1936.)

TABLE IX

The Interaction of Light Intensity and Nitrogen Supply in the Leaf Production of A. tenuis

Light treatments. Daylight.	Total leaf production (May 8–July 9, 1936) (gm. dry matter per pot).			
	Control.	Nitrogen treatments. (NH ₄) ₂ SO ₄ . Ca(NO ₃) ₂ .		Mean.
1.0 . . .	11.61	24.04	23.42	19.69
0.63 . . .	12.27	18.63	15.72	15.54
0.37 . . .	11.01	11.78	9.83	10.87
Mean	11.63	18.15	16.32	
Significant difference (i) between treatments				= 1.55
(P = 0.05) (ii) between means of three treatments				= 0.89

in leaf production. Inspection of Fig. 9 shows that while over the initial period extra nitrogen increased growth it decreased growth at the later stages. Thus in Table IX, where the total leaf production for the six cuts has been analysed,

the positive and negative effects tend to cancel out. This difficulty can be overcome by fitting straight line regressions. For the purpose of the statistical analysis, instead of fitting linear regressions direct to the leaf production data, the logarithmic function has been used. Such a transposition not only simplifies the calculation but also allows the various treatments to be compared independently. The regression coefficients of the logarithms with time are given in Table X. The analysis shows that in the absence of additional nitrogen there is no significant difference between the three light treatments. On the other hand, at the lowest light intensity the effects of ammonium sulphate and calcium nitrate are significant, the fall in leaf production with time being significantly greater in the case of calcium nitrate.

TABLE X

The Interaction of Light Intensity and Nitrogen Supply in the Leaf Production of A. tenuis

Treatments.		Regression coefficients of the logarithms of leaf production against time.
Light intensity.	Nitrogen supply.	
1.0 Daylight	Control	-0.0136
0.63 "	"	-0.0219
0.37 "	"	-0.0237
0.37 "	(NH ₄) ₂ SO ₄	-0.0412
0.37 "	Ca(NO ₃) ₂	-0.0653
Significant difference between treatments 0.0120		
(P = 0.05)		

As in the case of *A. tenuis*, the results for *T. repens* show a marked similarity to the effects observed in the pot experiment of the previous year. Reductions in the light intensity have led to large changes in leaf production, but additional nitrogen has had little effect (see Fig. 10). In fact, at each light level there are no significant differences in growth between the control pots and those receiving either ammonium sulphate or calcium nitrate (see Table XI).

TABLE XI

The Interaction of Light Intensity and Nitrogen Supply in the Leaf Production of T. repens

Light treatments.		Total leaf production (May 8-July 9, 1936) (gm. dry matter per pot).			
		Nitrogen treatments.			
Daylight.		Control.	(NH ₄) ₂ SO ₄ .	Ca(NO ₃) ₂ .	Mean.
1.0	. . .	17.27	18.52	18.12	17.97
0.63	. . .	10.98	12.51	11.83	11.77
0.37	. . .	7.53	7.59	6.64	7.25
	Mean	11.93	12.87	12.20	
Significant difference (i) between treatments		= 1.81			
(P = 0.05) (ii) between means of three treatments		= 1.04			

The relationship between the number of defoliations and the effects of light intensity on leaf production.

In several experiments it has been demonstrated that the effects of both light intensity and nitrogen supply become more pronounced as the number of defoliations increases. In order to facilitate comparison between the various

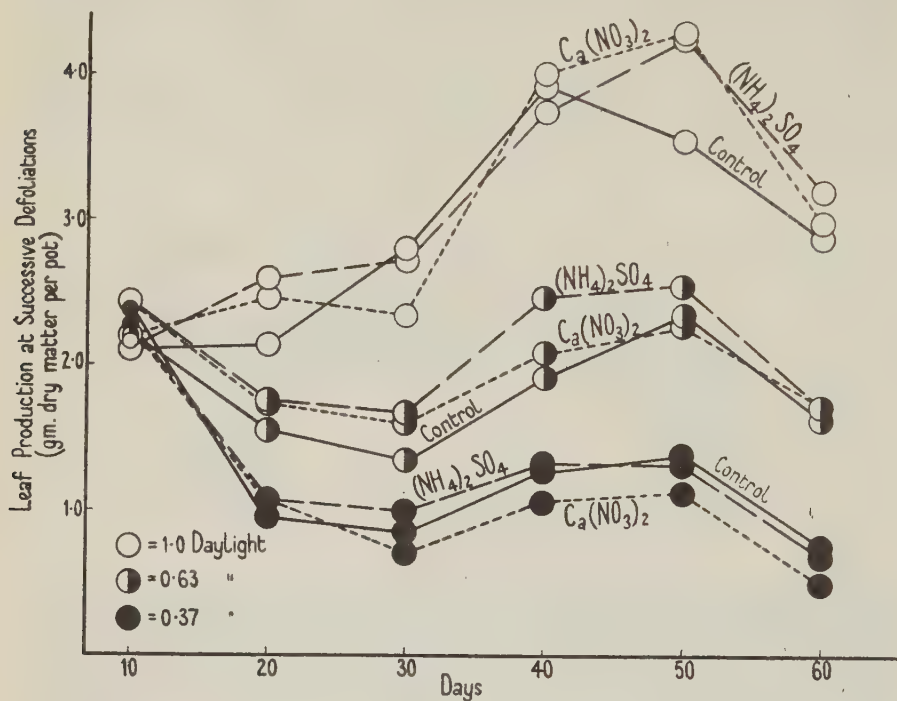


FIG. 10. The effects of light intensity and nitrogen supply on the leaf production of *Trifolium repens* when frequently defoliated. (May 8–July 9, 1936.)

defoliations and the several experiments in regard to the progressive light effect at each cut the amount of tissue regenerated in daylight has been taken as 100 for the individual manurial treatments and the comparable leaf productions at the lower light intensities expressed relative to this figure.

The clover results for the two 1935 experiments and the 1936 experiment shown in Fig. XI are in close agreement. For the period prior to the initial defoliation the effects of reduced light intensity were very different from those in subsequent periods. In the 1935 pot experiment a reduction to 0.61 of daylight first caused on the average a small but significant diminution in leaf regeneration, while a further reduction to 0.44 raised the leaf production to the same level as in daylight (see Table XII). In the 1935 field experiment, however, lowering the light intensity to 0.61 significantly increased the production of leaves for the first period, but a further reduction to 0.43 of daylight had no additional effect. On the other hand, in the 1936 experiment leaf

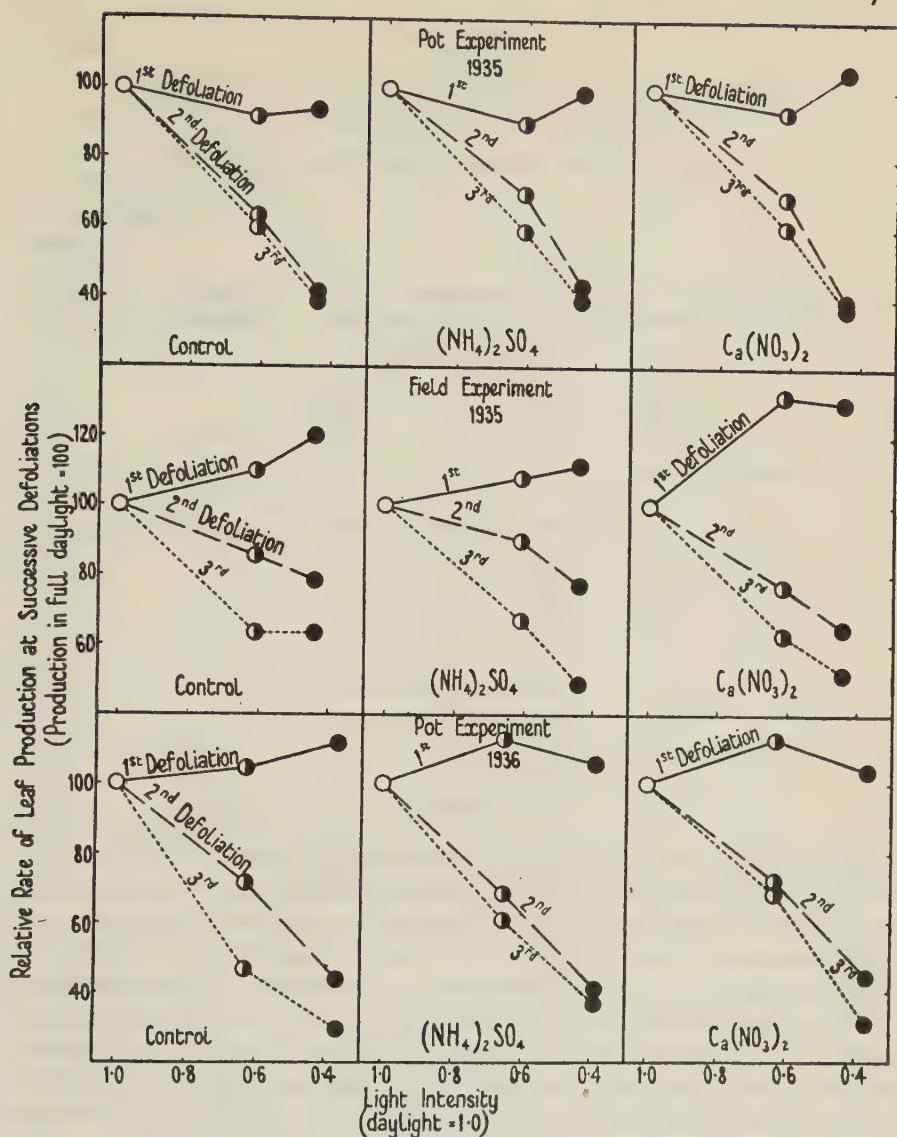


FIG. 11. The effects of light intensity on the leaf production of *Trifolium repens* at successive defoliations. At each defoliation and for each of the nitrogen treatments the leaf production in full daylight has been taken as 100.

production was not initially significantly affected by a decrease in light intensity (see Table XII). Subsequent to the first defoliation, in each of the experiments a fall in the light intensity was reflected in a marked diminution in leaf regeneration. Moreover, in two of the experiments (1935 and 1936 pot experiments) leaf production was almost directly proportional to the light intensity (see Fig. XI).

TABLE XII

The Interaction of Light Intensity and Nitrogen Supply in the Leaf Production of T. repens

Production of leaves for initial period (gm. dry matter).

Production of leaves per plant (gms.)						Significant difference between means of three treatments.
Light treatments.	Manurial treatments.				between treatments.	between means of three treatments.
Daylight.	Control.	(NH ₄) ₂ SO ₄ .	Ca(NO ₃) ₂ .	Mean.		
1935 Pot Experiment.						
1'0.	.	5'31	5'36	5'05	5'24	
0'61	.	4'83	4'79	4'73	4'78	
0'44	.	4'97	5'26	5'29	5'17	
	Mean	5'04	5'13	5'02	0'40	0'23
1935 Field Experiment.						
1'0.	.	25'8	28'0	25'6	26'5	
0'61	.	28'4	30'2	33'7	30'8	
0'44	.	31'3	31'2	33'3	31'9	
	Mean	28'5	29'8	30'9	6'5	3'8
1936 Pot Experiment.						
1'0.	.	2'10	2'15	2'19	2'15	
0'63	.	2'19	2'43	2'46	2'36	
0'37	.	2'38	2'28	2'25	2'30	
	Mean	2'22	2'29	2'30	0'42	0'24

The results of the *Agrostis* experiments show a marked similarity to those obtained for *T. repens*. In the 1935 pot experiment (see Fig. XII and Table XIII) a reduction in light intensity initially increased leaf regeneration, but in the subsequent cuts a decrease in intensity ran parallel with a falling off in leaf production. In the field experiment a diminution in light first significantly increased regeneration (see Table XIII). At the time of the second cut, however, a decrease in the light diminished leaf production not on the control but only where either ammonium sulphate or calcium nitrate had been added. This difference between the manured and unmanured plots indicates that subsequent to the first cut nitrogen rather than light intensity was the principal factor governing leaf production in the unmanured plots. There was a similar difference between the control and nitrogen treatments in the 1936 experiment. In the first period shading did not significantly affect the production of leaves except in the control at the level of 0'63 daylight (see Table XIII). In the period prior to the second cut in the controls changes in the light intensity were not reflected by differences in leaf production, but the nitrogen-treated pots showed a marked fall in production, especially when the intensity was reduced to 0'37 (see Table XIII).

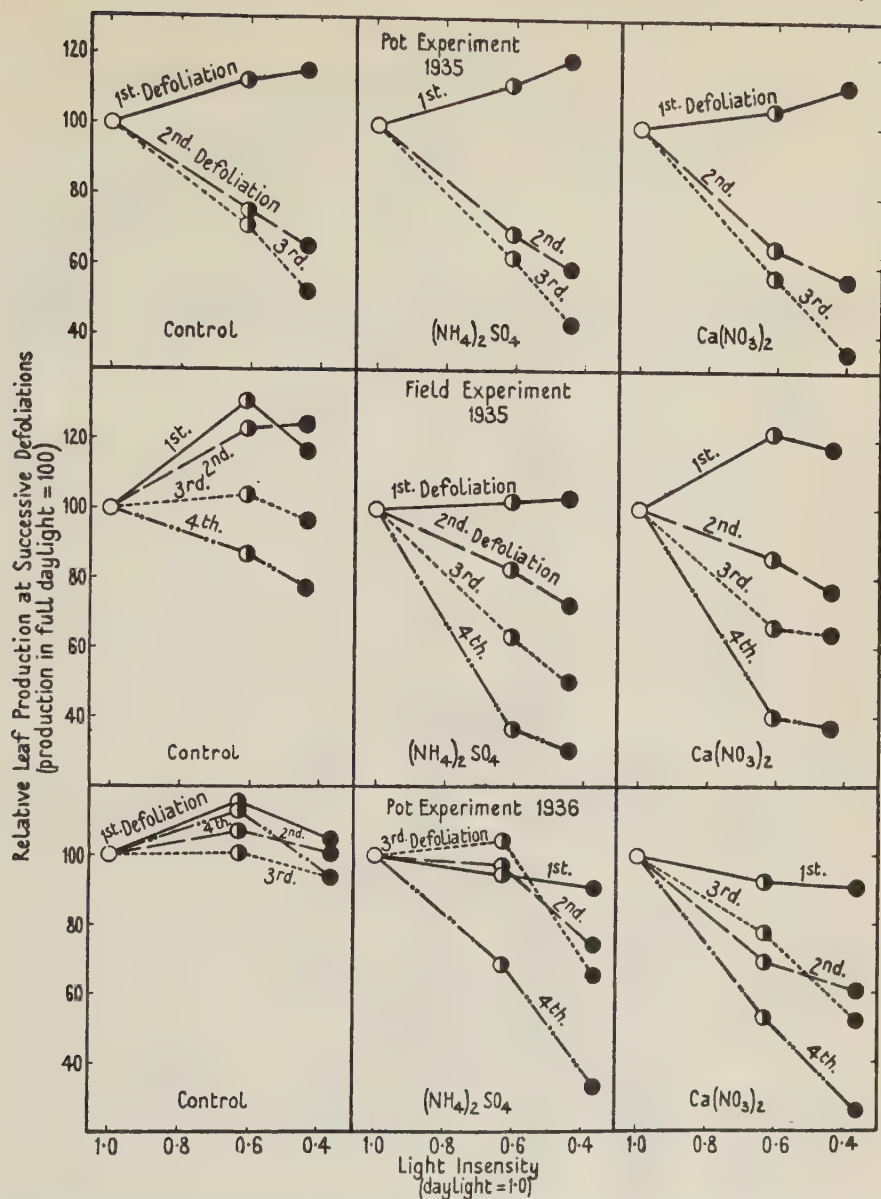


FIG. 12. The effects of light intensity on the leaf production of *Agrostis tenuis* at successive defoliations. At each defoliation and for each of the nitrogen treatments the leaf production in full daylight has been taken as 100.

The results obtained for *F. rubra* in the 1935 pot experiment are shown in Fig. XIII. It is seen that the behaviour of *F. rubra* was very similar to that of *A. tenuis*. Lowering the light intensity to 0.61 of daylight first

TABLE XIII

The Interaction of Light Intensity and Nitrogen Supply in the Leaf Production of A. tenuis

Production of leaves (gm. dry matter).

Production of leaves (gm. dry matter).						Significant difference between means of three treatments.
Light treatments.		Manurial treatments.				between treatments.
Daylight.		Control.	(NH ₄) ₂ SO ₄ .	Ca(NO ₃) ₂ .	Mean.	
1935 Pot Experiment.						
First defoliation.						
1.0.	.	2.13	2.21	2.11	2.15	
0.61	.	2.40	2.48	2.48	2.45	
0.44	.	2.46	2.63	2.47	2.52	
	Mean	2.33	2.44	2.35		0.32 0.19
1935 Field Experiment.						
First defoliation.						
1.0.	.	22.1	35.4	32.6	30.0	
0.61	.	29.2	36.2	39.9	35.1	
0.44	.	25.7	36.8	38.6	33.7	
	Mean	25.7	36.2	37.1		7.4 4.3
1935 Field Experiment.						
Second defoliation.						
1.0	.	14.8	33.5	32.3	26.9	
0.61	.	18.2	27.8	28.0	24.7	
0.44	.	18.3	24.2	25.1	22.6	
	Mean	17.1	28.5	28.4		4.1 2.4
1936 Pot Experiment.						
First defoliation.						
1.0.	.	3.46	4.59	4.55	4.20	
0.63	.	3.92	4.45	4.21	4.19	
0.37	.	3.61	4.15	4.19	3.98	
	Mean	3.66	4.39	4.32		0.45 0.26
1936 Pot Experiment.						
Second defoliation.						
1.0.	.	1.32	2.19	2.70	2.07	
0.63	.	1.41	2.14	1.87	1.81	
0.37	.	1.32	1.63	1.35	1.43	
	Mean	1.35	1.98	1.97		0.33 0.19

increased leaf production significantly (see Table XIV), but a further reduction had no effect. In direct contrast, a diminution in light intensity in the later cuts decreased leaf regeneration proportionately.

Effects of shading on soil temperature.

Since it was realized that reductions in the light intensity would probably be associated with temperature differences, the screens were placed some

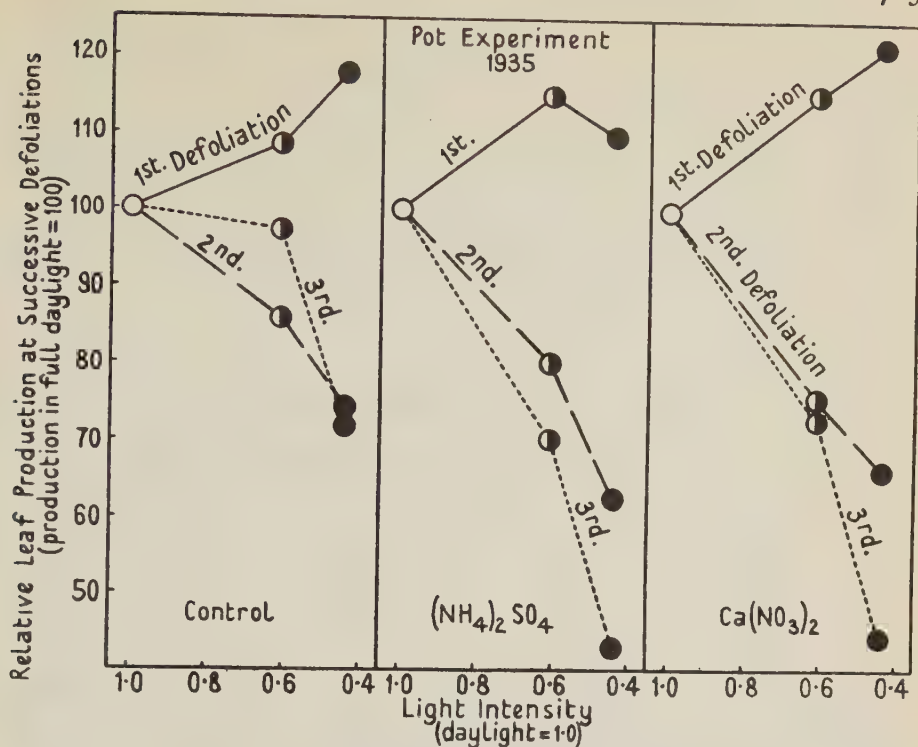


FIG. 13. The effects of light intensity on the leaf production of *Festuca rubra* at successive defoliations. At each cut and for each of the manurial treatments the leaf production in full daylight has been taken as 100.

TABLE XIV

The Interaction of Light Intensity and Nitrogen Supply in the Leaf Production of F. rubra

Light treatments. Daylight.	Production of leaves for initial period (gm. dry matter).			
	Control.	(NH ₄) ₂ SO ₄ .	Ca(NO ₃) ₂ .	Mean.
1.0	2.73	2.82	2.62	2.72
0.61	2.96	3.24	3.03	3.08
0.44	3.21	3.10	3.19	3.17
Mean	2.97	3.05	2.95	
Significant difference (i) between treatments				= 0.37
(P = 0.05) (ii) between means of three treatments				= 0.21

considerable distance above the surface of the pots in order to promote a free circulation of air. In several experiments thermometers with their bulbs suitably screened were suspended just above the surface of the pots and the temperatures recorded daily. The results showed that the air temperatures

under the screens were very similar to those in the open. On the other hand, thermometers placed in the soil at a depth of one or four inches demonstrated that screening the pot surface from direct insolation caused small temperature differences. The data for the 1934-6 groups of pot experiments are set out in Table XV. The results are very similar to those recorded in the field experiments (Blackman, 1938). At 9 a.m. the soil in the shaded pots is somewhat warmer owing presumably to the screens reducing the radiation of heat during the night. By 5 p.m., however, the position is reversed, since the exposed pots have attained a higher temperature than those which were screened.

TABLE XV
Effects of Shading on Soil Temperature

Year.	Light intensity. Daylight = 1·0.	Differences in temperature between shaded and unshaded pots. ° C.	
		9 a.m.	5 p.m.
1934	0·62	+0·21	-1·07
1935	0·61	+0·08	-1·13
1936	0·63	+0·49	-1·03
1935	0·44	+0·30	-1·41
1936	0·37	+0·27	-1·47

During the experimental periods the average 9 a.m. temperature was 16-18° C., and the 5 p.m. temperature 20-25° C. It seems therefore improbable that over the diurnal cycle a mean difference of less than 1° between the unshaded and shaded pots would favour to any appreciable extent the plants growing in full daylight. Even though the reductions in growth due to shading might be attributed in small part to the lower temperature, the differences in the effects of ammonium sulphate and calcium nitrate within various light levels are independent of such a factor.

DISCUSSION

In earlier experiments (Blackman, 1932, 1934) it was demonstrated that the repeated addition of ammonium sulphate to frequently defoliated swards decreased the content of species other than the grasses more than did calcium nitrate. At the time an explanation based on the work of Prianishnikov was put forward, that this difference between ammonium sulphate and calcium nitrate was due to a toxic accumulation of ammonium ions, occurring not in the grasses but in such species as *Trifolium repens*. It is not proposed in this paper to deal fully with Prianishnikov's results, since these have been discussed in earlier papers (Blackman, 1934, 1938) and in recent reviews of nitrogen metabolism by McKee (1937) and Nightingale (1937). Briefly, Prianishnikov demonstrated for a number of plants, including several legumes, that etiolated seedlings placed in solutions of ammonium salts were adversely affected. It was held that as soon as exhaustion of the available carbohydrate supply

checked further elaboration of the absorbed nitrogen, then ammonia nitrogen accumulated in toxic amounts. On this hypothesis it should follow that at low light intensities which limited carbon assimilation ammonium sulphate would have a greater effect on *T. repens* than at higher light intensities. Furthermore, as a corollary it was put forward that the difference between ammonium sulphate and calcium nitrate should come greater as the light intensity decreased. As it has been pointed out in the introduction, the field experiments described in the first paper of this series (Blackman, 1938) showed results exactly opposite to those expected from the earlier hypothesis.

On the basis of the present investigation the previous results can be accounted for by a more or less complete reversal of the initial assumptions. The balance of evidence clearly shows that at light intensities which limit growth, it is the grasses, not the clover, which are adversely affected by the addition of ammonium sulphate. In the three pot experiments of 1935 and 1936 it has been demonstrated that ammonium sulphate caused significant depressions in the leaf production of *Agrostis tenuis* and *Festuca rubra* at intensities of 0.44–0.37 daylight. Yet the growth of clover was unaffected by nitrogen in spite of the marked effect such light intensities had on growth. The results of the other comparable experiments showed, however, no deleterious effect of ammonium sulphate at any of the light intensities employed.

The present investigation has, however, revealed results even more unexpected than those already indicated, namely the interaction between light intensity and the effects of ammonium sulphate and calcium nitrate on the growth of the grasses. In the 1933 *F. rubra* experiment ammonium sulphate and calcium nitrate were not significantly different when the plants received normal daylight, yet at a light level of 0.6 daylight leaf production was significantly less when ammonium sulphate was added. Subsequent to 1933 the experiments gave different results, since at low light intensities the positions of ammonium sulphate and calcium nitrate were reversed. In the *F. rubra* experiments (1934 and 1935) ammonium sulphate relative to calcium nitrate tended to increase growth more at 0.61–0.62 than in full daylight. Furthermore, in 1935 only calcium nitrate significantly depressed growth at the light level of 0.44. In two out of three *A. tenuis* experiments this difference between the forms of nitrogen was most marked. In the 1935 pot experiment, although both ammonium sulphate and calcium nitrate largely increased leaf production in daylight, yet at the level of 0.61 only ammonium sulphate increased growth, while calcium nitrate reduced it. Similarly in 1936 ammonium sulphate and calcium nitrate in full daylight accelerated leaf growth equally, but at an intensity of 0.63, where light was in part a controlling factor, the response to ammonium sulphate was significantly greater. Moreover, at intensities of 0.44–0.37 calcium nitrate depressed growth more than ammonium sulphate in both experiments. On the other hand, in the 1935 field experiment there was no difference between the two forms of nitrogen at the three levels of light employed.

In contrast with the results obtained for the two grasses, in none of the clover experiments was any difference between ammonium sulphate and calcium nitrate dependent upon the light intensity. In two of the four 1934, 1935, and 1936 experiments the average effect of ammonium sulphate was significantly greater, but in none of the experiments, even at high light intensities, did additional nitrogen increase growth to the same extent as in the grasses.

In view of Prianishnikov's observations it is clear that these results cannot be adequately interpreted without a consideration of the effects of light intensity and nitrogen supply on the accumulation of the various nitrogen fractions within the plant. It is not proposed, therefore, to discuss these results in detail until the changes in protein synthesis and carbohydrate content have been described in subsequent papers. It will be shown that, unlike the clover, the grasses at low light intensities still actively absorb the nitrogen added either as ammonium sulphate or calcium nitrate. Under such conditions if there is a low carbohydrate content, the absorbed nitrogen is not synthesized into protein.

TABLE XVI

*Changes in Temperature and Light Intensity for the Periods
between Successive Defoliations*

	Average daily temperature (° C.)		Mean daily hours of sunshine.	Mean length of day.
	maximum.	minimum.		
<i>1935 Pot Experiments.</i>				
1st defoliation	74.3	55.7	7.86	16.63
2nd ,,	74.1	52.2	9.79	16.50
3rd ,,	79.9	52.4	10.65	16.26
<i>1935 Field Experiments.</i>				
1st defoliation	75.1	51.9	4.75	14.55
2nd ,,	72.6	52.6	4.97	14.20
3rd ,,	67.9	50.3	5.04	13.75
<i>1936 Pot Experiments.</i>				
1st defoliation	60.4	46.3	6.30	15.48
2nd ,,	61.1	41.3	6.37	15.97
3rd ,,	60.2	40.1	5.63	16.30

Apart from the interaction between light intensity and nitrogen supply, these experiments have also demonstrated that under a system of frequent defoliation, shading has markedly decreased growth both in the grasses and the clover. The investigations of other workers have not on the whole shown that comparable reductions in the light intensity have diminished growth to the same extent. The experiments, for example, of Popp (1926), Zillich

(1926), Shirley (1929, 1935), Scharrer and Schropp (1934), Clements and Long (1934), and Novikov (1936) indicate for many species, *Helianthus annuus* being an exception, that growth is decreased appreciably only below a level of 0.5 daylight. These investigations, however, differ essentially from the present experiments in that the plants were allowed to grow unchecked. It would be expected that the frequent removal of the leaves would render plants more susceptible to low light intensities. In this connexion it has already been demonstrated that the effect of shading is dependent upon the frequency of defoliation. In the case of the material removed at the first defoliation, a lower light intensity either increased or did not affect leaf production in the two 1935 and one 1936 clover experiments. Yet in the second and third periods, leaf production was almost directly proportional to the light intensity. The results for *A. tenuis* and *F. rubra* were essentially the same if allowance is made for the fact that nitrogen rather than light was the factor limiting the growth of those plants which did not receive additional nitrogen. Since these effects were common to the three species and to the several experiments, it seems unlikely that the failure of shading to depress leaf production in the first period was due to differences in light intensity between the first and subsequent periods. The data in Table XVI confirm this view, since the temperature, hours of sunshine, and length of day were not particularly favourable to growth in the initial periods. As alternative explanations there seem to be two possibilities. Since prior to the experimental period all the plants were allowed to grow in daylight without cutting, some reserves of carbohydrate would have accumulated in the roots and the basal portions of the shoot. On the plants being cut back, it is possible that, under reduced light intensities, but not in full daylight, these reserves are mobilized and utilized for leaf production. As a result of this shift in the carbohydrate balance, regeneration for the first period may be greater than in full daylight where the normal partition of the carbohydrates between the leaves and the rest of the plant operates. In the subsequent defoliations leaf production at low light intensities will be more dependent on the amount assimilated and less on these carbohydrate reserves. In the present experiments it would appear that the reserves are exhausted in the first two defoliations, since by the time of the third period leaf production is in general almost linearly proportional to the light intensity. There is also the possibility that apart from such reserves, some internal factor linked with the frequency of defoliation governs leaf production. It might be, for example, that at low light levels the photosynthetic efficiency decreases with the number of defoliations.

Finally, while in the present experiments a marked interaction between light intensity and nitrogen supply has been demonstrated for the two grasses investigated, it does not follow that plants less frequently defoliated will behave similarly. It is hoped in future experiments to determine how far the depressant effect of high nitrogen supply under low light intensities is dependent upon the interval between successive defoliations.

SUMMARY

In order to study further the parts played by light intensity and nitrogen supply in the competition between grasses and clover eleven pot experiments and two field experiments were carried out from 1933 to 1936. In ten experiments the plants were grown in soil, but in the remaining three a sand-bentonite mixture was employed. Differences in light intensity were maintained by shading the pots or plots with one to three layers of butter muslin stretched on wooden frames. Additional nitrogen, either as ammonium sulphate or calcium nitrate, was added every 7–10 days at rates equivalent to 37·5–50·0 lb. nitrogen per acre. Prior to the experimental period the plants were allowed to grow unchecked in full daylight for at least six weeks, but during the experimental period they were defoliated at frequent intervals by cutting with shears.

In the case of the grasses, i.e. *Agrostis tenuis* and *Festuca rubra*, the effects of both ammonium sulphate and calcium nitrate were dependent upon the light intensity. With full daylight the response to additional nitrogen was marked; in two out of five experiments ammonium sulphate caused a larger increase. Lowering the intensity to 0·63–0·6 of daylight accentuated this difference between the two forms of nitrogen, for in four out of the five experiments the results were significantly different. In one instance calcium nitrate was superior to ammonium sulphate, but in three other experiments the increase due to ammonium sulphate was significantly greater. Moreover, in one experiment, although ammonium sulphate increased leaf production, calcium nitrate depressed it. When the light intensity was further reduced to 0·44–0·37 then both ammonium sulphate and calcium nitrate reduced leaf production in three out of four experiments. In addition, the depression brought about by calcium nitrate was significantly greater.

In direct contrast with the results obtained for the grasses, there was no evidence with *Trifolium repens* that differences in the effects of ammonium sulphate and calcium nitrate were dependent upon the light intensity. In daylight additional nitrogen caused a small rise in leaf production (three out of four experiments), while at intensities of 0·63–0·6 the nitrogen effect was even less marked. At the lowest light level (0·44–0·37) there was no response to nitrogen, neither was there any indication that either ammonium sulphate or calcium nitrate depressed leaf growth.

In general, the response of all species both to light intensity and nitrogen supply was dependent upon the number of defoliations. At each light level the effects of additional nitrogen became progressively greater with successive defoliations. On the other hand, reductions in the light intensity gave results at the time of the first cut which differed from those in subsequent cuts. For the first period shading *T. repens* either increased or did not affect leaf production, but in the following defoliations leaf production was almost directly proportional to the light intensity. When the grasses were supplied with

additional nitrogen, the results showed the same trend as in the case of *T. repens*. In some experiments, however, in the absence of nitrogenous manuring the light effect was masked, since growth was limited by nitrogen deficiency.

It is concluded from this and the previous investigation (Blackman, 1938) that in grassland communities the balance between legumes and grasses is dependent largely upon competition for light. When the sward is infrequently defoliated then the taller growing grasses shade the clovers. Since the density and height of the grasses is correlated with the nitrogen supply, additional nitrogen may depress the leguminous species merely by increasing the degree of shading without any direct effect of nitrogen on the legumes.

The authors are indebted to Imperial Chemical Industries for permission to publish the results of the experiments jointly carried out in 1933-5. They also wish to thank Dr. M. S. Bartlett for his help in the statistical interpretation of the data.

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Sampling as the Cause of the Apparent Growth Cycles of *Lemna minor*

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With eight Figures in the Text

I. INTRODUCTION

IN a previous paper (Dickson, 1938*a*) it was demonstrated that measurements of the growth of *Lemna minor* showed two cycles. One of these had a periodicity of four to six days, the other of twenty-five to forty days. In each case it was shown that the duration of a complete cycle was probably a function of the rate of growth of the plants. It was also observed that the wave-phase sometimes changed when a colony was transferred to a different environment, and that the extent of the alteration was probably determined by the particular phase at the time of transfer. It was suggested that the cycles may be related to the fact that the growth data (frond counts and dry weight determinations) are those of colonies of numerous individuals, and that the cycles, while exhibited by the colonies are not shown by the individual plants. This view has been followed up by experiment and a theory advanced to account for the presence of the cycles.

II. EXPERIMENTAL RESULTS

The general cultural conditions employed are similar to those described in a previous paper (Dickson, 1938*b*) and will not be repeated here. Where there are differences in detail for the several experiments they are set out below, together with the results. Certain of the data are taken from experiments described in the earlier paper.

a. The effect on the wave-phase of a change in environment.

In the previous paper it was pointed out that the differences between the regression coefficients of certain replicate experiments (K, L, M, and N) were partly due to the relative rate of frond division being faster at the beginning of the experiments than subsequently, and also to differences in the length of the several experiments. An effect due to the wave-cycle of the parent colony, experiment E, was also involved and it was stated that this effect would be dealt with in a later paper. Fig. 1 shows part of the wave-cycle of the parent

colony, experiment E, growing in twelve-hour alternations of light and darkness, and also the curves of log. frond number with time of experiments K, L, M, and N growing in one-minute alternations. The plants for these four experiments were taken from the parent colony on January 27, February 5, &c. Straight lines have been drawn through the first two points of each curve to show the initial slopes.

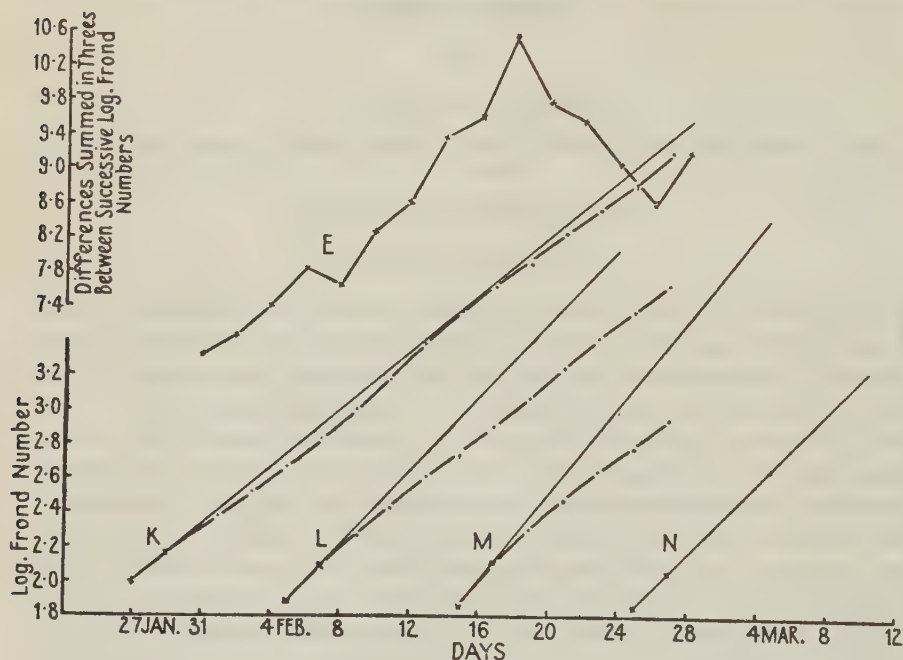


FIG. 1. The four curves K, L, M, and N are of colonies derived from samples taken successively from the parent colony of Experiment E. Straight lines are drawn through the first two points of each curve to show the initial slopes.

emphasize the differences between the rates of increase of log. frond number in the first two days and the rates subsequently. It will be seen that the rate for the first two days is greatest in experiment M, somewhat less in L, and lowest in K and N. After the first two days the rates in all cases decrease and approach a common rate. A comparison of the initial rates with the wave-cycle of colony E, shows that as the parent colony approaches the crest of its wave so the initial rates increase and are lower again after E has passed its maximum point. While this result has not been repeated it is thought probable that the particular phase of the parent colony, at the time material is taken, has an effect on the initial rate of growth of the sample in its changed environment. It also appears probable that the extent of this initial 'stimulus' has a bearing on the wave-phase of the new colony, as it has been shown (Dickson, 1938a, fig. 1) that one daughter colony may have a wave-phase the inverse of that of its parent, while a second one, taken subsequently when the parent colony was in a different phase, may show the same phase as its parent.

b. Experiments on the interaction of the plants in a colony.

Two experiments were designed to show whether the waves in growth measurements could be attributed to a 'social biological' effect, i.e. to some form of interaction between the different plants in a colony. In the first of these, two colonies were grown, each in its own dish of culture solution and

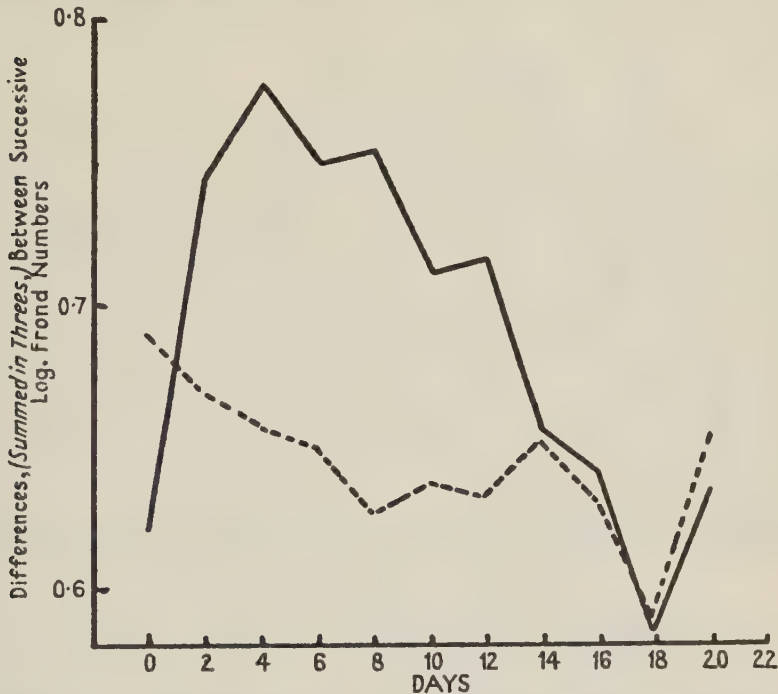


FIG. 2. Growth curves of two colonies of *Lemna* in each of which individual plants were grown in separate supplies of culture solution.

their wave-phases, which were approximately the inverse of one another, determined. They were then placed in the same dish but kept distinct by floating each colony within a wax ring. Frond counts were then continued. No change was found in the waves of either colony which indicates that plants in one wave-phase have no effect on the phase of other plants grown in the same medium.

The second experiment was designed to show whether a number of plants grown and measured under standard conditions, but with each plant having a separate supply of culture solution, would show a cycle in growth measurements. For this purpose a number of small specimen tubes were packed into a dish and held in position with paraffin wax. They were filled with solution and one plant was placed in each. Every second day the plants were removed, washed, and the number of fronds determined. One plant was then replaced in each tube and the surplus discarded. Fig. 2 shows the results of two such

sets. It will be seen that in one set there is a well-defined wave, while in the other the result is indefinite. It is concluded then, that there is no evidence for the existence of a 'social' biological effect and that a wave can be produced where no reaction is possible between the members of a colony.

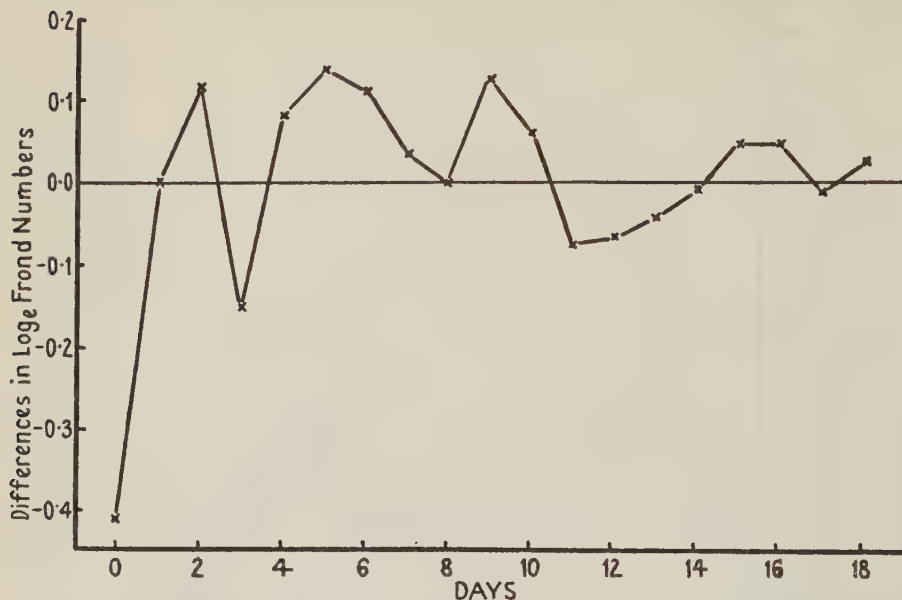


FIG. 3. Growth curve of a colony grown from a single frond without sampling, i.e. without removing any fronds.

c. The wave-cycles of a colony grown from a single frond without sampling.

A single immature frond was selected, placed in a dish with culture solution and allowed to grow for nineteen days, a count of the total number of fronds produced being made every day. No fronds were removed from the colony throughout the experiment. The results are shown in Fig. 3 in which the differences between successive log. frond numbers and their respective values calculated from the line of closest fit are plotted against time. A wave, having a short periodicity of from four to five days is present, but this decreases in amplitude as the colony gets larger until it has almost disappeared by the end of the experiment. Two other determinations similar to the above were made with the same results. It appears evident, therefore, that while many of the colonies in which frond numbers have been determined and which in most if not all cases show the presence of a short-wave cycle (*vide* Dickson, 1938a, Figs. 3 and 4; Ashby, 1929, Fig. 8, and Ashby and Oxley, 1935, Fig. 41),¹ have

¹ In the case of the last two references the experiments were too short to make the existence of a short wave certain, but it is definitely suggested by several of the curves.

originated more or less recently from single fronds, this method of forming a colony cannot be held responsible for the origin of the short wave.

The experiment was not continued for sufficiently long to determine whether a long-wave cycle is present or not. An analysis of the curve shows a slight falling off in rate of division with time. This is but to be expected, however, for as the experiment proceeds the number of older fronds increases, and Winter (1937) has shown that as a *Lemna* frond ages so the intervals between the production of successive daughter fronds increase.

d. The effect of a long period of darkness on the short-wave cycle.

An alternative method of producing a colony of *Lemna* plants to that of allowing a single frond to multiply, and one which is not uncommonly used, is to take a sample of plants from some stock source, where they are generally in an inactive condition, and grow them under standard conditions for two or three weeks before beginning measurements. It appeared possible that fronds of a random sample of plants taken from a source where growth was not active and transferred to conditions where rapid division was possible, might start to divide at approximately the same time and so set up a rhythm in the frond counts. The method of division of a frond is complex; first it divides to give a frond on the left side (as viewed with the rounded end pointing from one), then one on the right. The 'left-hand' frond matures first and divides successively on the left and right, and similarly with the right-hand frond. Winter (1937) has shown that each frond continues to divide until ten or twelve daughter fronds have been formed, and then dies.

In view of this complicated form of division it was decided to determine only the interval between the first appearance of a 'left-hand' frond and the appearance of the latter's first left-hand daughter; in this way the question of interpreting the results was much simplified. The following method was adopted. Fifty-one plants were selected, each plant consisting of a frond with a young left-hand daughter frond. The plants were divided into three sets of seventeen plants each, and each was placed in a hole punched in waxed paper discs floating in culture solution. They were examined daily and the solution was then changed. The first appearance of a left-hand daughter frond was noted for each plant and also the occasion on which this daughter frond gave rise to its first daughter, and so on. It was found that as soon as a daughter frond had been produced the parent frond could be readily separated from the parent plant which was then discarded. This process was carried out daily with each plant for twenty-nine days. Two of the three sets of plants were then placed in the dark in an incubator at 23° C. for seven and eleven days respectively, during which time the solutions were only changed once. They were then returned to their previous environment and divisions noted on the following four days. In this way, *with the exception of the period in the incubator*, the plants had been examined every twenty-four hours. Fig. 4 shows the results for each set. The numbers of new fronds formed on any one day

were added together for each set and are shown plotted against time. It will be seen that in each case a definite wave is established with a periodicity of about four days, this is attributed to the fact that the daughter fronds with

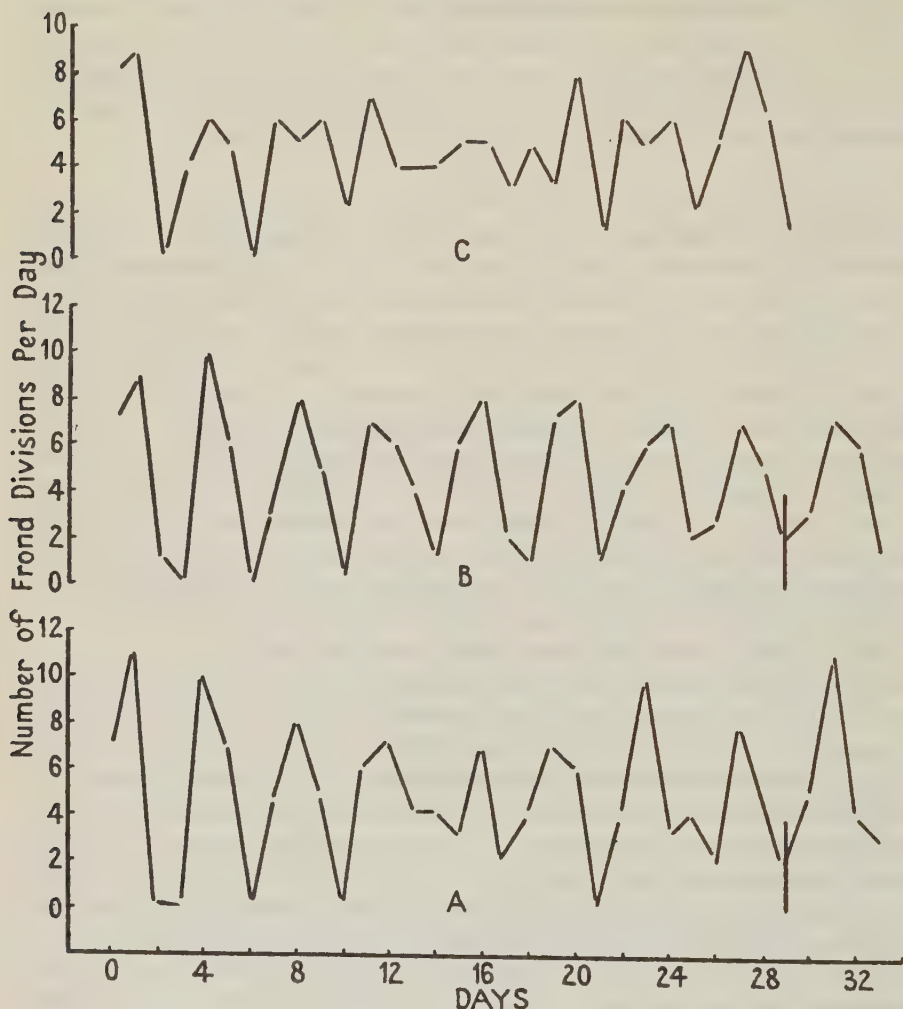


FIG. 4. The vertical lines in the curves A and B represent periods of seven and eleven days respectively during which the plants were kept in the dark.

which the experiment was begun were not a random selection but were in most cases of about the same size, and would therefore be expected to give rise to new fronds at about the same time. The mean calculated time interval (total days/number of divisions) between the births of successive daughter fronds for the three sets is 3.74 ± 0.03 days. As, however, the plants were not observed continuously this means that on some occasions there will have been

an interval of three, and on others of four days, between divisions. The ratio of the number of three-day to four-day divisions calculated from this mean will therefore be $26/74$ or $1/2.85$. The results of the dark period in the sets A and B are shown in Fig. 4. The vertical lines on the twenty-ninth day represent the dark periods of respectively seven and eleven days. It will be seen that the plants behaved exactly as if there had been no dark period, growth being resumed at the normal rate immediately the original conditions were restored. An analysis of division times of the individual plants showed that with very few exceptions divisions occurred at times which the results previous to the dark period would lead one to expect. In some of the exceptional cases division occurred a day early, in others a day late. It is concluded from these results that plants, following a period under conditions which prevent active division, resume their growth according to their previous individual stages of development. There is no tendency for, say, young fronds to divide prematurely and so get into step with others which are on the point of division. It may be taken as generally improbable then that a cycle can be established by transferring a sample of fronds from a poor environment to one that favours active growth.

It is of interest here to note that the set of fronds after eleven days in complete darkness were a little paler than a comparable set which had been continuously in the light, but that otherwise they appeared perfectly normal. The loss of 'roots', which is an early and therefore sensitive indication of an unsuitable environment, did not take place during the period of darkness.

e. The effect of sampling on the short-wave cycle.

Two colonies of *Lemna* A and B were established under continuous light at a temperature of 26°C . The number of fronds in each colony was counted every second day for a period of thirty-four days. During the first twenty-four days the colonies were 'sampled' after each count, i.e. the fronds were reduced to a constant number, in this case to 45. For the remainder of the time no fronds were removed from the colonies. The results are plotted in Fig. 5, in which the differences between successive log. frond numbers and their respective values calculated from the lines of closest fit are plotted against time. It will be seen that in each colony a short wave with a periodicity of about seven days is present from the beginning of the experiment to the twenty-fourth day when sampling was discontinued. After the twenty-fourth day the amplitude of the wave is greatly reduced. On the thirty-fourth day each colony consisted of some 1,100 fronds, so it was not practicable to continue the experiment further. It is considered that these results, together with those of the experiment described in Section II, *c*, indicate that sampling and the short-wave cycle are intimately connected, and that on the cessation of sampling any wave which may be present at the time gradually decreases in amplitude and probably ultimately disappears. As in all these determinations of the rate of increase of frond number during which sampling has been carried

out regularly and for a sufficiently long period a short-wave cycle has been found, it is considered justifiable to conclude that the short wave is maintained and is probably initiated by the act of sampling. An explanation of how sampling gives rise to and maintains a wave in growth measurements is put forward in the discussion.

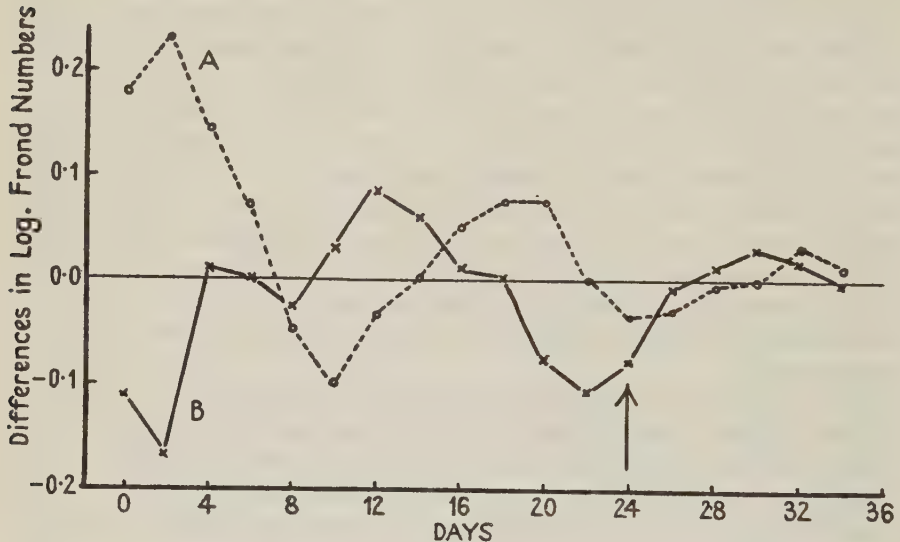


FIG. 5. Curves showing the effect of sampling on the short-wave cycle. After the first twenty-four days the colonies were counted every two days but were allowed to grow without reduction of number.

III. DISCUSSION

Short waves.

It has been shown that the short wave is not due to the methods generally used in starting a colony of *Lemna* plants, i.e. either by multiplication from a single frond or by transferring relatively inactive plants to conditions where rapid division is possible. Neither is the wave due to any effect of one individual of a colony upon another. The experiment described in Section II, *c*, during which there was no sampling, and in which a wave of short period was observed to decrease in amplitude until it almost disappeared by the end of the experiment, together with the experiments of Section II, *e*, indicate that the sampling process is closely related to the existence of the short wave and it is believed that this process not only gives rise to the wave but also maintains it. In order to illustrate the effect of sampling one may consider the results of sampling on the rate of division of 400 fronds selected at random from a normal population. Assume that the time from the first appearance of one frond to that of its daughter is four days, that counts are made every day, and that following each day's count the total number of fronds is reduced by random sampling to the original number of 400. Since the first sample is a

random one the probability is that 100 fronds will divide each day. The table shows the estimated results based on these assumptions.

TABLE I

Original fronds.	Count 1st day	Number reduced to 400.	Count 2nd day.	Number reduced to 400.	Count 3rd day.	Number reduced to 400.	Count 4th day.	Number reduced to 400.
100	200	160	160	133.3	133.3	114.5	114.5	100
100	100	80	160	133.3	133.3	114.5	114.5	100
100	100	80	80	66.6	133.3	114.5	114.5	100
100	100	80	80	66.6	66.6	57.3	114.5	100
Total frond numbers. } —	500	—	480	—	466.6	—	458.0	—

The numbers of fronds occurring on successive days will then be 500, 480, 467, 458, 500, &c. Thus it is seen that assuming regularity of division and sampling a short wave is set up with a periodicity which is a function of the rate of frond division. While it is realized that the effects of the different rates of division of right- and left-hand fronds and of old and young fronds, and the complications introduced by the relatively long period between the birth of a frond and the occasion of its first division have been neglected in this calculation it is nevertheless considered from the evidence that sampling in the manner described is entirely responsible for the presence of the short wave. It is probable that once the colony has come into equilibrium with its environment the mean rate of division of the colony in the absence of sampling, is constant. The example quoted above is also exceptionally simple in that the period of division is a simple multiple of the interval between counts. Assuming that 3.7 days—the interval found by experiment in Section II, d , is the division period for every frond, and that measurements are made on alternate days, then each frond and its descendants will be observed to divide on the following days:—every fourth day for six divisions, one division after two days, every fourth day for six divisions, one division after two days, every fourth day for five divisions, then one division after two days; this gives a total of seventy-four days and completes the cycle. A simpler case has been worked out in full for a random sample, where the division period is 3.5 days and measurements are made on alternate days. The complete cycle occupies fourteen days in this case, and two cycles are shown in detail in Fig. 6. The condition here is the same as that which occurred in the experiment described in Section II, d , except that in the latter case the cycle was longer owing to the different division rate (3.74 days) and that counts were made daily.

Long waves.

The presence of a long-wave cycle has been demonstrated by the author in eight separate instances (Dickson, 1938*a*), and it has been shown that as a result of the wave measurements based on observations extending over seven days may differ from the mean rate by as much as 20 per cent., and where

two rates are compared with colonies in opposite phase a difference of 40 per cent. may arise from the wave effect alone. Significant differences between adjacent up and down slopes have been demonstrated by Student's method (Fisher, 1928) in two experiments (E and H) in the above paper. In one case where $n = 4$, P was less than 0.02 and in the other where $n = 11$ the difference was of even higher significance. Owing to the comparatively short duration of most experiments on *Lemna* few of the published results could be

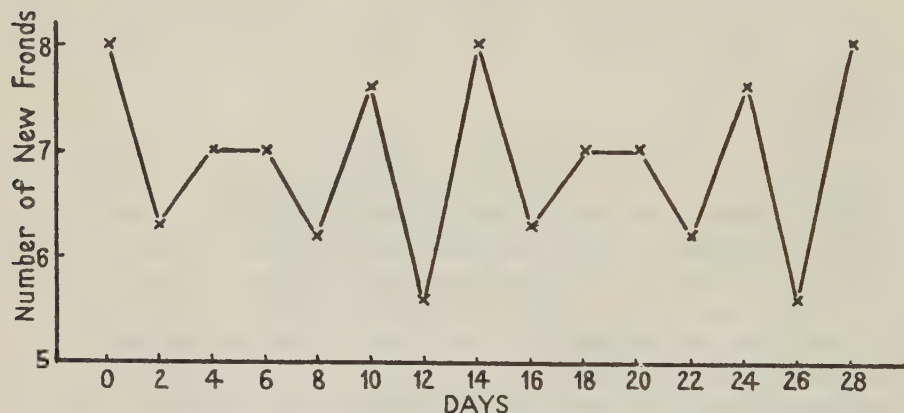


FIG. 6. Theoretical curve showing two complete cycles. For explanation see text.

expected to show the long wave. In an experiment by White (1936, Fig. 1), however, in which experiments were continued for eighteen days, two of the three curves illustrated show a long regular variation which might well be part of a long wave.

A sample taken from a colony has been found to have a wave not always in phase with that of the parent colony and it has been shown that the wave-phase of the sample is probably dependent on the particular phase of the colony at the time of its removal. Alteration of the frequency of sampling also probably has an effect on the long wave (Dickson, 1938a, Fig. 2, experiment E). The experiment described in Section II, *b*, of this paper shows that, as in the case of the short one, the long wave is not due to any 'social' effect, i.e. of one individual on another.

Several factors have been considered as being possible causes of the long-wave cycle. In the first place the irregularity in division rates calculated from counts made only at fixed intervals suggested a possible source of the rhythm. Fig. 6 shows that this factor may in effect introduce a long cycle, but its amplitude is necessarily less than that of the short waves, whereas in actual measurements it is much greater. Secondly, two different rates of division such as those of left-hand and right-hand fronds, when imposed on one another will give a 'beat' of longer period than that of either of its components. Such a possibility is illustrated in Fig. 7, where two sine waves of different period are shown, together with the curve resulting from the

addition of their values at regular intervals of time. Again, the amplitude of any wave so formed cannot exceed that of the two waves taken together and

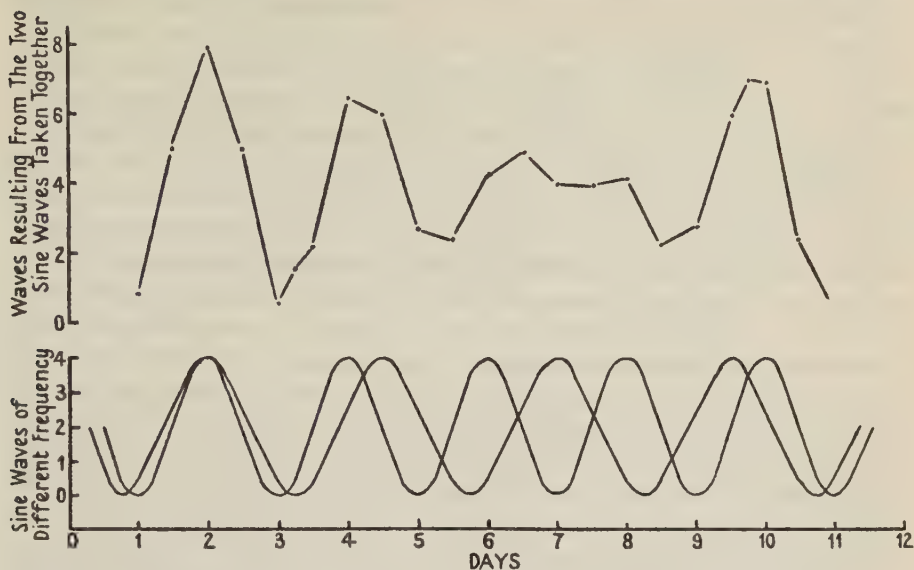


FIG. 7. Two sine waves of different frequency and the wave resulting from the addition of their values at regular intervals of time.

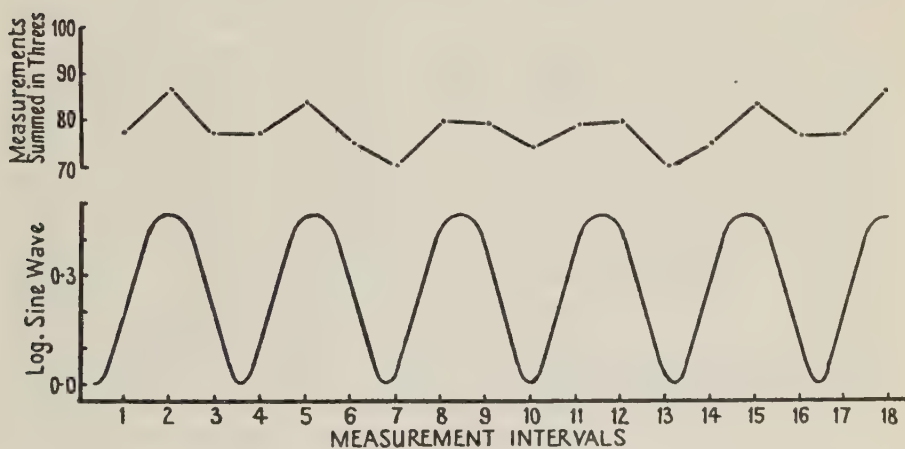


FIG. 8. For explanation see text.

a variation of this order is not sufficient. Fig. 8 shows the logs. of the values of a sine wave, together with the curve resulting from measurements taken at fixed intervals along it. The ratio of the intervals to the period of the sine wave was $1.25/4$. It will be seen that a curve so formed shows not only a short wave but also a long one; as in the previous two cases, however, the amplitude

of the latter cannot exceed that of the former and consequently is not sufficient as an explanation of the present case. It is therefore evident that none of these factors can be responsible for the origin of the long wave. It is considered that the most probable cause of the long wave, i.e. the rhythm in frond number counts, is the effect of sampling on the relative numbers of young and old fronds present in a colony, and that the length and magnitude of the wave so formed is dependent on the frequency and manner of sampling in relation to the division rate. The effect of random sampling (carried out in the case of *Lemna* in order to keep the number of fronds in a colony from becoming unmanageably large) in an established colony consisting of fronds of all ages, is gradually to remove those fronds which are slowest in dividing, i.e. old mature fronds and young immature fronds. By doing so the average division rate for the colony is increased and the up slope of a wave initiated. After this process has continued for some time the removal of immature fronds will begin to have an effect in causing a dearth of mature young and rapidly dividing fronds with a consequent decrease in the division rate. As the division rate decreases so does the severity of sampling (the percentage of fronds removed per day is smaller), with the result that fewer immature fronds as well as fewer older fronds are removed, a further decrease in the division rate therefore results and the down slope of the curve is initiated. This hypothesis as to the origin of the long-wave cycle also explains the action of the various factors which have been found to affect the wave. For example, it offers a ready interpretation as to why the observed frond-division rate is a function of the wave period, and the variation in the average frond weight is related to the wave-phase, and it explains a change in the long wave on altering the frequency of sampling. An alteration of the mean division rate by transferring a colony to new conditions while the period of sampling remains unchanged will obviously have an effect on the wave-cycle, and it can also be seen that the phase at the time of removal to the new conditions may be expected to have a bearing on the phase in which the colony starts in its new environment.

As already pointed out, the error introduced into a determination of the mean rate of increase in frond number over a period of seven or eight days, may be as much as 20 per cent. above or below the true mean, an error, due entirely to the existence of the long-wave cycle. An error of this size will not be of frequent occurrence (unless the period during which measurements are made is reduced) but smaller variations from the mean rate will occur more often, so that it is obviously necessary to adopt some modification of the method usually employed whereby the occurrence of the long wave and consequently of such errors can be eliminated. While this could be achieved by merely ceasing to sample, such a solution would prove impracticable owing to the very large numbers of fronds which would ultimately have to be dealt with. It is suggested that the method adopted in Section II, *d*, in which the average interval between the first appearance of a left-hand daughter frond and that of its first left-hand daughter was determined would prove most

satisfactory. It would not be necessary, as in the experiment described to keep the different plants separate, all could be grown together as is the normal practice. The advantages of this method are as follows: (a) Sampling affects only those fronds which are no longer involved in the count, so that a short-wave cycle will not be initiated; (b) as all fronds involved in the estimation throughout the experiment will be of the same age, and also since sampling does not affect such fronds, no long-wave cycle will arise in the measurements; (c) it is probable that plants will come into equilibrium with a new environment sooner than was the case when using the original method, as the only fronds concerned in estimating the division rate are young ones; (d) the labour involved in a determination of the rate of frond increase should be no larger than that in the present procedure.

IV. SUMMARY

Experiments are described which indicate that the short-wave cycles (Dickson, 1938a) found when the frond number is determined by the usual methods are not the result of the particular method adopted when first establishing a colony of *Lemna*.

By growing plants each in a separate tube it has been demonstrated that the long wave is not due to any 'social biological' effect, as under these conditions a well-developed wave cycle was formed.

It has been shown both theoretically and by experiment, that the presence of the short wave is due simply to the process of sampling in general use, i.e. to the regular reduction in the number of fronds which is necessary to keep the number within practical limits. Thus it has been shown in the absence of sampling that no short wave is to be found, but that it appears as soon as sampling is begun. Theoretically it has been shown that a short period wave will arise in a random collection of fronds as a result of sampling.

Various methods by which the long-wave cycle might be expected to be formed are discussed, but in each case the maximum amplitude of the waves so produced was never sufficiently large. Finally, it is proposed that the effect of sampling in removing fronds which are slow in dividing (especially newly formed immature fronds) offers an adequate explanation of the existence of the long-wave cycle. This view is supported by a number of observations which have been made concerning changes in sampling methods and growth conditions.

In conclusion I wish to express my indebtedness to both Professor V. H. Blackman and Professor F. G. Gregory for many helpful suggestions and criticisms throughout the course of these experiments.

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NOTES

THE EMBRYO AND GAMETOPHYTE OF PSILOTUM TRIQUE-TRUM. A Preliminary Note.—As the result of field investigations on this plant extending over a number of years, the writer has discovered a large number of gametophytes. Two facts of special interest have come to light which seem to warrant immediate publication in the form of a preliminary notice. A full account, based on a considerable amount of material, is in course of preparation, and it is the writer's intention to submit this for publication in the near future.

The Embryo and Young Sporeling

A sufficient number of developmental stages, from the fertilized egg on to the detached young sporeling, have been obtained to make it clear that the embryogeny of *Psilotum* is essentially the same as that of *Tmesipteris*. The embryo of *Psilotum* consists of two organs only, viz. shoot and foot, the latter having the same haustorial processes as in *Tmesipteris*. There is no trace of either root or cotyledon.

The special interest of this similarity lies, of course, in the remarkable simplicity of organization which is now known to characterize the embryo in both genera. The facts which have come to light with respect to *Psilotum* can be held to increase the possibility that the existing *Psilotales* have retained a more or less primitive organization. The comparison of this group with the Devonian *Psilophytales* has thus an added interest.

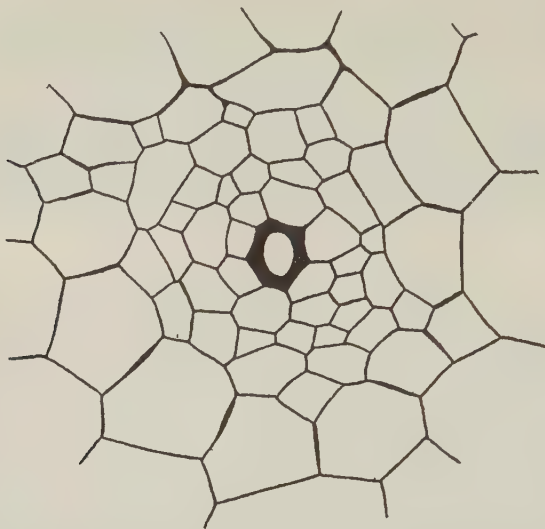
A Vascular Strand in the Gametophyte

The gametophyte of *Psilotum* grows to a larger size than that of *Tmesipteris*. The writer has found incomplete specimens up to 20 mm. in length, and 2 mm. in diameter. These are probably several years old. In his original account of this gametophyte (*Trans. Roy. Soc. Edin.*, lii. 1917) Lawson figures an object which had a total length of nearly 20 mm. and a diameter of just under 1 mm.

A number of portions of gametophytes found by the present writer are from $1\frac{1}{2}$ to 2 mm. in diameter, and are thus of much stronger growth than those found by Lawson. In these there is a well-defined conducting strand which, in the rather older regions of the gametophyte, possesses in some cases one or two tracheides (see Figure on page 808). These thick-walled tracheides are invariably situated at the centre of the strand, and are completely surrounded by a zone, 2 to 4 cells in width, of narrow, thin-walled elements with dense contents, which can be styled 'phloem'. In regions of the gametophyte where this conducting strand is best developed it is surrounded by a very distinct endodermis. The endodermal cells show typical thickenings on the radial walls, and sometimes also at the cell angles, these thickenings staining very strongly with phloroglucin or safranin, as do also the tracheides. In microtomed longitudinal sections the tracheides are seen to be typically annular. The phloem elements are elongated with oblique end walls, but sieve areas have not been seen on them.

This strand, or stele as it may be termed, is very similar in general structure to

that of very young sporophytes of *Tmesipteris* (and presumably of *Psilotum* also). It differs from the latter, however, in certain important particulars. It has not been seen to originate from the apex of the gametophyte, but first appears in a slender form at a considerable distance behind the apex. It extends forward as the



Psilotum triquetrum. Transverse section of hinder region of large gametophyte showing well-defined stele with one tracheid, phloem zone, and endodermis. (Camera lucida drawing $\times 285$.)

gametophyte grows in length. When traced back into progressively older regions it is found invariably to be discontinuous in character, fading out and reappearing again a little farther back. In one case this fading out and reappearing was found to take place three times in a length of 5 mm.

Wherever the strand is well formed it is invariably surrounded by a fungus-free zone, 2 to 4 cells in width, packed with starch, the strand itself showing neither fungus nor starch. Where the strand fades out the central tissues are uniform in character with those of the gametophyte as a whole, and are filled with fungal coils.

The writer has not found any indication whatever of a conducting strand in any of the largest gametophytes of *Tmesipteris* examined by him. Nor is there any indication of it in smaller gametophytes of *Psilotum*, even when these are bearing an embryo or a young sporeling. Lawson does not refer to such a strand in either *Tmesipteris* or *Psilotum* (*Trans. Roy. Soc. Edin.*, vols. li, lii, 1917).

It may be added that the *Psilotum* objects with a strand possessed in every case also numerous antheridia and archegonia covering the surface more or less closely. In some cases one or more embryos were also present on these same objects.

It is not intended to discuss these facts at any length in the present communication. It will suffice here to indicate briefly three possible ways of interpreting this gametophytic stele. There is the possibility, firstly, that these large gametophytes possessing a conducting strand are peculiar instances of phenomena known in apogamy and apospory, but there seems to be no direct support for such an explanation in this case. Secondly, there is the suggestion that the strand may have merely a physiological significance. Its invariable relation to the nearby

accumulation of starch might be taken as corroborating this view. The possibility that typical annular tracheides and a typical endodermis can arise spontaneously in a gametophyte seems to carry with it far-reaching conclusions with respect to both the phylogenetic and the ontogenetic origin of conducting tissues in the various organs of the sporophyte of vascular plants. Thirdly, there is the possible explanation that the conducting strand in the *Psilotum* gametophyte is vestigial in character. This interpretation involves the conception of the homologous origin of the alternation of generations in Pteridophyta. It certainly agrees with the facts that the gametophyte and the young sporophyte, in both genera of the Psilotales, are in various respects remarkably alike one another, and that this group of Pteridophyta is commonly regarded as representing a very archaic stock.

JOHN E. HOLLOWAY.

DUNEDIN, N.Z. 1

CAUSE OF THE DISAPPEARANCE OF THE CYCADEOIDEA IN THE CRETACEOUS PERIOD.—No phanerogams have been recorded before the Lower Cretaceous period when, in Europe and North America at least, the greater part of the flora consisted of Cycadaceae, Ginkgoaceae, Coniferae, and ferns; but in the Lower Cretaceous period a number of angiosperms appear, increasing in the Upper Cretaceous era, while at the same time the Cycads become more scanty and have disappeared from the north temperate regions entirely in the Eocene era. The cause of this sudden change has been variously explained by botanists. K. Fujii (Bot. Mag. Tokio, xxiv. 213) suggests, as factors, attacks by fungi, injurious gases from volcanoes, and change of climate. Attacks by fungi may be eliminated, for though it is possible that a species has been locally so destroyed, it is clearly impossible for a whole flora of very wide distribution to be thus exterminated. Volcanic gases as a cause is also an impossibility. Apart from the fact that there is no evidence of extensive volcanic activity in the area affected, every one who has botanized on and round volcanoes, say in Java and Sumatra, will recognize the fact that the effusion of gases has practically no effect on the flora on their slopes, which is often very rich and old. Change of climate, which Fujii considers the most probable cause, would hardly be likely to affect more than a comparatively small area, and not the whole world. K. Yendoh suggests that the evolution of insect pollinators may have played an important part, but that would hardly account for the rapid disappearance of the Cycads alone. Groom (Ann. Bot., 1910, xxiv. 268) suggests that conifers are more easily deranged and killed by sudden injuries and are attacked by more serious fungal and insect foes than dicotylous trees. I hardly think this is so, and certainly the Palms, which depend for their existence on a single bud, came strongly to the front in Upper Cretaceous and Eocene days.

My suggestion is that the rapidly growing broad-leaved trees, Magnolia, Artocarpus, Oak, Plane, Credneria, Populus, and laurels, with their quick development, densely spreading masses of foliage, and rapid and abundant seed-production, shaded out the slow-growing Cycads and gymnosperms with their narrow leaves, by cutting off their light supply and probably their water supply as well, just as the bracken eliminates the heather and grass on our open hills, and *Brachypodium sylvaticum* the small herbaceous plants of our downs. Besides this the close growth of the trees and thick foliage would prevent the dispersal of pollen and effectual

fertilization. Fujii says that the range of wind-fertilization of Cycads is 100 metres, and I can confirm the very short distance apart required for the pollen of the male plant to reach the female, from notes made in the Botanic Gardens of Singapore.

In some parts of the Malay Peninsula Cycads, apparently *C. Rumphiana*, are to be found persisting in dense forests, usually on limestone rocks, and now far remote from the sandy shores of sea or river which is their normal habitat. There is evidence, however, that at no very long period of time the sea or some river had been close to these rocks. Only on very rare occasions do these plants ever produce carpophylls. Indeed, I have only once seen these, and never a male cone. The plants reproduce solely by bulbils which fall off and rolling down the banks give rise to adults. Such Cycads as did not produce bulbils would disappear very speedily under an invasion of rain forest, and even those which did could only remain for a short period.

The Christmas Island Cycas growing on open rocks produced a few bulbils, but it seems to be dying out now, for Andrews in 1897 describes it as growing all round the island and most plentiful on the south coast, but in 1904 I found very few of these plants left.

The Cycads of the present day are nearly all found in hot, dry places, sea-shores, limestone rocks, or open veldt, very seldom in shade. They are of exceedingly slow growth: Chamberlain ('The Living Cycads') gives an instance of *Encephalartos Altensteinii* growing 1 foot in forty-six years, and one, a century old, being only 6 feet high. Most of these plants never attain a height of more than 6 feet, though the *Dioon spinulosum* of Mexico is recorded as tall as 50 feet, and *Macrozamia Moorei* 60 feet in height.

Till the evolution of the big angiosperm trees no rain forest could have existed, and it was the invasion of the lofty, rapidly reproducing, forest trees and shrubs which exterminated the old Cycadoid flora. In the rain forest region the only Coniferae to be seen are the broad-leaved *Dammara*s and *Podocarpus Blumei* Endl. and these scantily. The narrow-leaved species of *Podocarpus* and *Dacrydium* are confined to sea-shores or the high mountains, where the big trees of the rain forest do not occur, and where it is possible for them to obtain sufficient sunlight and diffuse their pollen to the female flowers. The evolution of the broad-leaved dense forest is, I think, enough to account for the disappearance of the Mesozoic Cycads and gymnosperms.

H. N. RIDLEY.

The Constancy of the Mean Net Assimilation Rate and its Ecological Importance

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THE work of Gregory (1926) showed that in barley, up to the time of maximum leaf area which coincides approximately with the first differentiation of flowers, the net assimilation rate was independent of time. It was also found to be unaffected by a fourfold increase in nitrogen supply. During this first part of the growth cycle the net assimilation rate fluctuated about a mean value, over 80 per cent. of the variation being accounted for by the variation in external factors. More recently Heath, working in South Africa (1937*a*, 1937*b*), found that in the cotton plant the net assimilation rate had no general trend in time during the vegetative phase but fluctuated about a mean rate. The results of Crowther (1934) for cotton in the Sudan also appear to show no drift of net assimilation rate with time until after flowering has begun. The data of Williams (1936) for oats indicate that 'unit leaf rate' (i.e. net assimilation rate) has no drift in time for the first four to six weeks. On the other hand, experiments with Sudan Grass by Ballard and Petrie (1936) show a fall in unit leaf rate from the first observation at 2 weeks (*vide* Williams, 1937, Heath, 1938). The data of Watson and Baptiste (1938) for mangolds sown on six different dates show a single high initial value after which the net assimilation rate has no marked trend for a period of at least ten weeks. Their results for sugar beet in the same experiment are similar, but show, for some sowing dates, two high values at the beginning. Considering every sowing for both crops, the high values mentioned occur, with one exception, in the periods June 26–July 10 and July 10–24. This strongly suggests that they were due to particularly favourable meteorological conditions during those periods, although there is no evidence as to the particular environmental factor concerned.

If the constancy of the net assimilation rate during the vegetative phase of annual plants, apart from fluctuations in rate due to meteorological conditions, is of general occurrence, a comparison of the mean rates for different species and environments should be of considerable ecological interest. On making

such a comparison it is found that there is extraordinarily little variation in the mean net assimilation rate for the early part of the life cycle for a number of very different species of plants in the most diverse environments.

The mean value over four seasons obtained by Gregory (1926) for barley growing out of doors in pots at Rothamsted was 0.546 gm. dry matter per square decimetre of leaf surface per week, the means for the separate seasons ranging from 0.42 to 0.66. Heath's results (1937*b*) for cotton (tops only) in South Africa were obtained on a leaf weight basis. They have, however, been converted to a leaf area basis, making use of the mean weight per square dm. over the appropriate time from sowing obtained from data presented by Portsmouth (1937) for the Sudan. After conversion the mean rates up to first flowering for the 'C' treatment are 0.567 and 0.418 gm. per square dm. per week for 1933-4 and 1934-5 respectively. It should be noted, however, that in the latter season the mean rate was much lowered by poor growth associated with aphid attack at one end of the experiment. The 'C' plot at the best end of the field, which was free from aphid, gives a mean rate of 0.521. A similar conversion has been carried out on Crowther's (1934) data for cotton in the Sudan. The means over all the nine treatments, which had no effect on net assimilation rate, for the period up to first flowering have been converted to a leaf area basis, again using Portsmouth's data. The value obtained for mean net assimilation rate is 0.685 gm. per square dm. per week.

F. J. Richards (unpublished data) determined the net assimilation rates during the early growth of three grasses very thickly sown. The rates in the fully manured series were lower than in the nitrogen deficient series, probably owing to the greater leaf growth and consequently greater degree of shading. Richards (personal communication) points out that in the fully manured series the dense mats of fine grass must also have effectively reduced the CO₂ supply to the leaves in the interior. For these reasons, only the nitrogen deficient series are considered here, but even in these the rates were low, probably owing to the dense stand. The mean rates for the three species were 0.323, 0.242, and 0.403.

Hornberger's data for leaf area and dry weight of *Sinapis alba*, quoted by Boysen-Jensen (1932), yield a mean rate for the five weeks up to time of maximum leaf area of 0.506 gm. per square dm. per week, the actual weekly rates varying between 0.67 and 0.31.

Watson and Baptiste (1938) determined the net assimilation rates of sugar beet and mangolds over fourteen-daily intervals for six different sowing dates ranging from April 9 to June 18. For the sake of brevity the results from the three earlier sowing dates have been averaged and also those from the three later ones. In each case all the data up to October 2 have been included. After this date there is a marked fall in net assimilation in many of the series.

Gregory's experiments on cucumbers grown under glass (Gregory, 1917, 1921) were carried out at three times of the year: November-December, February-March, and June-July. In the first two experiments, which gave

mean rates of 0.125 and 0.338 respectively, the low light intensity was probably limiting assimilation, as Gregory (1917) himself points out in relation to the first set. On the other hand, some of the results obtained by Bolas *et alia* (1938) for tomato seedlings under glass are almost as high as the usual value for outdoor plants in spite of short days and low light intensity. They carried out a number of experiments of short duration (seven hours) on dates between August 22 and October 14. The mean light intensity during the different experiments varied from 193 to 812 foot-candles, and the day lengths from 14½ hours on August 22 to 10¾ hours on October 14. Light intensity differences appear to have had little effect on the net assimilation rates recalculated as gm. per sq. dm. per week,¹ but there is a definite fall with day length. The mean values from the experiments in August, September, and October are 0.614, 0.468, and 0.421 respectively.

All the results referred to above are summarized in the Table. It will be noted that excluding the winter-grown cucumbers and the crowded grasses, the differences in net assimilation rate between different crops and environments are scarcely greater than those due to seasonal effects alone, as shown by Gregory's data for barley and Heath's for cotton. It may therefore be stated with some confidence that all the plants in their wide range of environments show practically the same mean net assimilation rate during the vegetative phase, although the nature of the data precludes the satisfactory application of statistical analysis.

This conclusion is strengthened by unpublished data for a bulbous perennial, *Scilla nutans*, obtained by G. E. Blackman. The mean net assimilation rate for flowering bulbs growing in full daylight, calculated for the period April 5, 1938 to April 29, 1938, i.e. from leaf expansion up to anthesis, was 0.615 gm. per square dm. per week. The mean day length for the period was fourteen hours.

DISCUSSION

It is clearly of the greatest importance for ecology to evaluate the assimilation rates of the various plants constituting a natural association. The method of estimating net assimilation rate was described and the theoretical importance of this measure was explicitly stated by Gregory (1917) in the following terms: 'This method gives us a ready means of obtaining the average rate of assimilation over long periods of time and at different seasons of the year.' The appropriate methods of growth analysis were formulated by Briggs, Kidd, and West (1920) and applied to Kreusler's data for maize, and the same methods have been used by Gregory (1921, 1926, 1928) in the study of growth of cucumber and barley. Among the derived growth data used was an estimate of carbon assimilation (unit leaf rate, net assimilation rate). In spite of this fact, however, singularly little use has been made of the methods of growth analysis for the solution of ecological problems. In America, for instance, a

¹ This calculation neglects losses due to respiration during the night.

Mean Net Assimilation Rates during Vegetative Phase—gm./dm.²/week

Plant.	Place.	Year.	Dates.	Mean day length (approx.).	Net assimilation.	Source.
Barley	Rothamsted	1921	10/5-28/6	16½	0.559	Gregory
"	"	1922A	17/5-5/7	16½	0.658	"
"	"	1922B	22/5-10/7	16½	0.642	"
"	"	1923	7/5-25/6	16½	0.418	"
"	"	1924	23/4-18/6	15½	0.452	"
"	"	1921-4	May-June	16½	0.546	"
Cotton (tops)	Barberton, S. Africa	1933-4	20/11-15/1	13½	0.567	Heath
"	"	1934-5	17/12-11/2	13½	0.418 (0.521)	"
Cotton (tops)	Gezira, Sudan	1928	8/10-18/11	11½	0.685	Crowther
Italian Rye grass	Rothamsted	1929	17/6-29/7	16½	0.323	Richards
Cocksfoot grass	"	1929	17/6-29/7	16½	0.242	"
Rough-stalked meadow grass	"	1929	1/7-29/7	16½	0.403	"
Sinapis alba	Bonn, Germany	1885?	19/5-23/6	16	0.506	Hornberger
Mangolds (I-III sowings)	Rothamsted	1934	26/6-2/10	14½	0.514	Watson and Baptiste
(IV-VI sowings)	"	"	10/7-2/10	14½	0.688	"
Sugar beet (I-III sowings)	"	"	26/6-2/10	14½	0.643	"
(IV-VI sowings)	"	"	10/7-2/10	14½	0.720	"
Cucumber	Cheshunt (glass)	1916	10/11-9/12	8½	0.125	Gregory
"	"	1917	21/2-22/3	11½	0.338	"
"	"	1917	11/6-10/7	16½	0.413	"
Tomato	"	1932	22/8 and 31/8	14	0.614	Bolas
"	"	"	19/9 and 21/9	12½	0.468	"
"	"	"	7/10, 13/10, and 14/10	10½	0.421	"

very large body of data has been accumulated in the endeavour to utilize the growth of standard plants as phytometers for the evaluation of the total effect of the climatic environment (Clements and Goldsmith, 1924; Clements, Weaver, and Hanson, 1929; Clements and Long, 1934, 1935). An examination of these data by the present authors showed that much of the information which might have been gained from these experiments was not forthcoming owing, presumably, to a lack of appreciation of the possibilities of growth analysis.

Again, on the Continent, the study of 'Trockensubstanzproduktion'

initiated by Boysen-Jensen (1918) has been confined to an estimation of percentage increment in dry weight of the plant per day either by direct estimation, using a discontinuous compound interest formula, or by calculation from estimations of CO_2 uptake of detached leaves combined with respiration rates of detached leaves, stems, and roots (Müller 1932). The limitations of such methods in comparison with the methods of growth analysis considered above should be sufficiently patent. Indeed, it is questionable whether such 'Substanzquotienten' give any satisfactory estimations of dry weight accumulation which can be used in ecological studies, and this for two reasons. The first is that the dry weight increment of plants is dependent on two factors (*a*) net assimilation rate or unit leaf rate and (*b*) the rate of growth of the leaf surface. The 'Substanzquotient' entirely neglects the second aspect of dry weight increase. The data presented in this paper are of primary interest in showing that net assimilation rate in the most diverse types of plant under very various conditions approximates to a constant mean value; of this later. Clearly, therefore, the main determining factor leading to very great differences in dry weight accumulation in different types of plants is the rate of extension of the leaf surface, in which is included both the size of individual leaves and the rate of production of new leaves. Even within a single species consideration of this aspect of dry weight accumulation is of the greatest importance. Thus as Gregory (1926) has shown the greater dry weight accumulation in the barley plant following on nitrogen manuring is due solely to the increase in the leaf surface thus induced and not to a change in net assimilation rate (see Table p. 828). That this factor of nitrogen supply is equally important in general ecological studies is manifest.

The second objection is that the estimation of assimilation rate by CO_2 uptake always involves enclosing the leaf within a chamber and maintaining a supply of CO_2 by passing a current of air over the leaf. Some of the difficulties involved in this procedure have been stressed by Boysen-Jensen (1928) who suggested new methods to circumvent them. Even so, the supply of CO_2 cannot be directly compared with that under natural conditions, and therefore estimations of assimilation thus obtained can be utilized only with circumspection. The point here to be emphasized, as Gregory (1937) has stressed, is that the methods of growth analysis supply estimates of net assimilation rate which are superior to those obtained by any other method, for the estimation of net gain of carbon *under natural conditions*.

Reviewing as a whole the data presented in the table it appears that the mean net assimilation rate in nature is no very variable quantity. Thus, comparing the mean value for barley over four seasons with the average rate for all other species, the values obtained in the two cases are 0.546 and 0.552 respectively. It would appear therefore that, *on the average*, despite variations in leaf structure in the plants investigated *the net amount of CO_2 utilized per unit area is everywhere the same*. The variations encountered among the different types of plants might, in the absence of further knowledge, be ascribed to such

variation in leaf structure, but the results for barley clearly show that such an explanation is not tenable, for the data here refer to the same plant. The variation in net assimilation rate within this species ranges from 0.418 to 0.658, whereas among the other species the extremes are 0.413 and 0.720, which is scarcely greater. Furthermore, among the barley series are included two in which the nitrogen supply was deliberately varied fourfold, thus resulting in leaves of very different morphological structure and chlorophyll content without, however, any difference in net assimilation rate. It does not therefore appear that leaf structure is necessarily a determining factor.

The variations in net assimilation rate in the barley experiments have been shown to be highly correlated with intensity of the external factors of light and day temperature, both of which control, to an almost equal extent, rate of assimilation. Since in nature these factors are highly correlated they may for the purpose of this discussion be considered together. Evidence may be brought forward to show that increase in CO_2 supply also leads to increase in assimilation in the case of barley, and this is well known to be the case in other species also grown under natural conditions (Bornemann, 1923; Lundegårdh 1924). It appears, therefore, that the constant value of net assimilation rate is not due to the fact that any one of the external factors is completely limiting the process, nor from the range of plants considered would it appear probable that internal factors are in all cases the same. This greatly limits the possible explanations of the results here presented. It is most unlikely that the results are purely due to chance. As stated above, the fact which emerges is that in all species and environments the same net amount of CO_2 is utilized per unit area. For such a result to obtain there must exist some measure of compensation for the effects of variations in leaf structure as between the different species and in day length and light intensity as between the different environments.

As far as different species growing in approximately the same environment are concerned (barley, mangolds, and sugar beet at Rothamsted), it seems profitless, in the absence of experimental evidence, to suggest what the mechanism of the compensation for differences in leaf structure might be. The same obtains with regard to the barley grown with high and low nitrogen (Gregory, 1926 and 1922A and 1922B of table) in which marked differences in leaf structure and chlorophyll content occurred.

The observed effects of light intensity and CO_2 supply upon a single species in a single environment (barley at Rothamsted) would be accounted for if, with the usual CO_2 content of the air, CO_2 supply were limiting net assimilation rate at or near the highest observed value (say about 1.2 gm. per square dm. per week). This would give a fairly high correlation with light intensity for net assimilation rates up to that value, although the curve would not be really linear. Also, as most values would be fairly near to the CO_2 limited value of net assimilation, increasing CO_2 would increase net assimilation. Considering now a shorter day, such as that in the Sudan or South Africa,

a lower mean net assimilation rate might be expected. But the shorter day could be compensated for by (a) higher mean light intensity or (b) by differences in leaf structure such as more stomata per unit area, &c. With regard to (a) it is noteworthy that Shirley (1935) states that during the growing season the amount of light received in twenty-four hours is similar in the tropics, the temperate regions, and the arctic regions. A possible explanation therefore of the remarkable similarity of net assimilation rates in Europe and Africa may be that the total quantity of light received is the limiting factor in all cases. The results of Bolas *et alia* (1938) for tomatoes grown under glass appear to refute, however, the suggestion of such invariable light limitation, for here low light intensity coupled with a short day has given net assimilation rates as high as those obtained for outdoor plants. It must be admitted that these results as here presented are over-estimated to an unknown extent, owing to the fact, already stated, that in conversion to a weekly basis losses due to respiration during the night periods have been neglected. Nevertheless, it appears most improbable that in Africa total light is limiting, and it seems more likely that there exists some other form of compensation whereby the plants are adapted to produce in all environments approximately the same quantity of dry matter per unit leaf-area. The factor that seems most likely to be near a limiting value in *all* natural environments is the quantity of CO_2 which in unit time can diffuse into the leaf and thence into the actual assimilating cells. Various modifications of leaf structure, such as differences in number of stomata per unit area or alterations in resistance of the cells to diffusion of CO_2 , might act as compensating factors for different day lengths so that in the shorter days more CO_2 reached the chloroplasts in unit time.

Whatever the mechanism responsible, it is a very remarkable fact which needs further investigation that plants as different as are barley, mangolds, cotton, and tomato, and in such diverse environments as out of doors in England and in Africa and under glass in England should all have the same mean net assimilation rate during their vegetative phase. That this should be so is indicative of the fundamental significance of the net assimilation rate. This is obviously the only really satisfactory measure of assimilation from an ecological point of view since it integrates the gains due to assimilation and the losses due to respiration of the whole plant under the actual growing conditions and the result is obtained without interference with the normal functioning of the leaves.

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Observations on the Response of Leaves of *Limnanthemum* and *Tropaeolum* to Light and Gravity

BY

W. NEILSON JONES

With three Figures in the Text

THE leaves of plants, as is well known, usually respond to unilateral illumination by a growth curvature of the petiole such as to bring the upper surface of the lamina perpendicular to and facing the light. Leaves are also sensitive to the stimulus of gravity, the position of equilibrium being usually with the lamina horizontal. When a leaf is subjected to the combined stimuli of light and gravity the position assumed finally is a compromise between the effects of these two—and any other—orientating stimuli.

The following observations record an attempt to evaluate the relative importance of light and gravity in the orientation of detached leaves of *Limnanthemum peltatum* and *Tropaeolum majus*.

It may be mentioned that attention was directed to these reactions by the curvatures sometimes shown by the petioles of leaves of *Limnanthemum* floating in a dish as part of a floral decoration. Such curvatures appeared to bear no relation to the direction of the incident light, but rather to whether the leaves had room in the dish in which they were floated to lie horizontally on the surface of the water. For study of the comparative behaviour of aerial leaves *Tropaeolum* was chosen, since this plant has leaves of somewhat similar form.

Limnanthemum peltatum.

Experiment 1. Detached leaves of *Limnanthemum* were floated in a tank which stood a little way from a window, the light striking the leaves at an angle of about 45° above the horizontal. The horizontally floating laminae were held in various positions with regard to the incident light by threading them on a cotton stretched across the tank at the level of the water surface. After $2\frac{1}{2}$ days' illumination little change was noted in the positions in which the petioles were attached to the laminae, any slight changes being quite unrelated to the direction of the incident light.

It would appear, therefore, that curvature of the petioles is not induced to any appreciable extent by unilateral illumination of the lamina, and that consequently light has little if any orientating effect on these leaves.

Experiment 2. In another series of experiments a sheet of cork faced with

filter-paper was fixed approximately vertical in a glass tank. Water was poured into the tank to form a shallow layer at the bottom, the lower edge of the cork sheet dipping into the water; the top of the tank was covered with a sheet of glass. A hole was bored in the cork sheet through which was passed the petiole of a leaf, the under surface of the leaf being in contact with the wet filter-paper. This arrangement was found to maintain the leaf in a fully turgid condition with the lamina in a vertical plane and the petiole free to move. The tank was then placed in a dark room in which the only source of light was from a lamp below the level of the tank, the rays of light from this lamp impinging on the upper surface of the leaf at an angle of about 45° below the horizontal.

If the curvature of the petiole is controlled chiefly by light, a downward curvature of the petiole is to be expected, such as would bring the lamina to face the light were the base of the petiole fixed and the lamina free to move; if gravity is the more effective stimulus, the petiole would curve upwards thus bringing the lamina, under natural conditions, into a horizontal position.

The results obtained in the case of three leaves in such an experiment are shown graphically in Fig. 1, which represents tracings of actual leaves during the course of an experiment. Evidently the leaf of *Limnanthemum* is extremely sensitive to gravity, a marked curvature being apparent after three hours. As in the former experiment, no evidence of any light stimulus is apparent.

Experiment 3. In a further experiment, instead of being in a saturated atmosphere, leaves were totally submerged in water with the laminae held vertically. Here also it was found that in whatever way the laminae were orientated in a vertical plane—with the tips of the leaves upwards, to the side, or pointing downwards—the petioles reacted by curving upwards.

From these results it appears that in *Limnanthemum* the reaction of the petioles is controlled, mainly at least, by gravity.

Tropaeolum majus.

Parallel experiments to those performed with *L. peltatum* leaves were carried out with detached leaves of *T. majus* and gave the following results.

Experiment 4. Leaves were floated in a tank and illuminated by light incident at 45° above the horizontal as in experiment 1. At the end of $2\frac{1}{2}$ days, whatever the position of the horizontal laminae in relation to the incident light, all the petioles had bent strongly towards the light, the curvature being in some cases more than 360° . Tracings from representative leaves in this experiment are shown in Fig. 2.

When the tip of the leaf is towards the source of light a simple bending occurs; when the side or base of the leaf is towards the light the movement appears to be more complicated and to involve a twisting of the petiole, causing it to move in a widely sweeping lateral curve to attain the final position.

Thus *T. majus*, unlike *L. peltatum*, shows a marked sensitivity to the direction of the incident light, when, as under the above conditions with leaf lamina horizontal, the stimulus due to gravity is at a minimum.

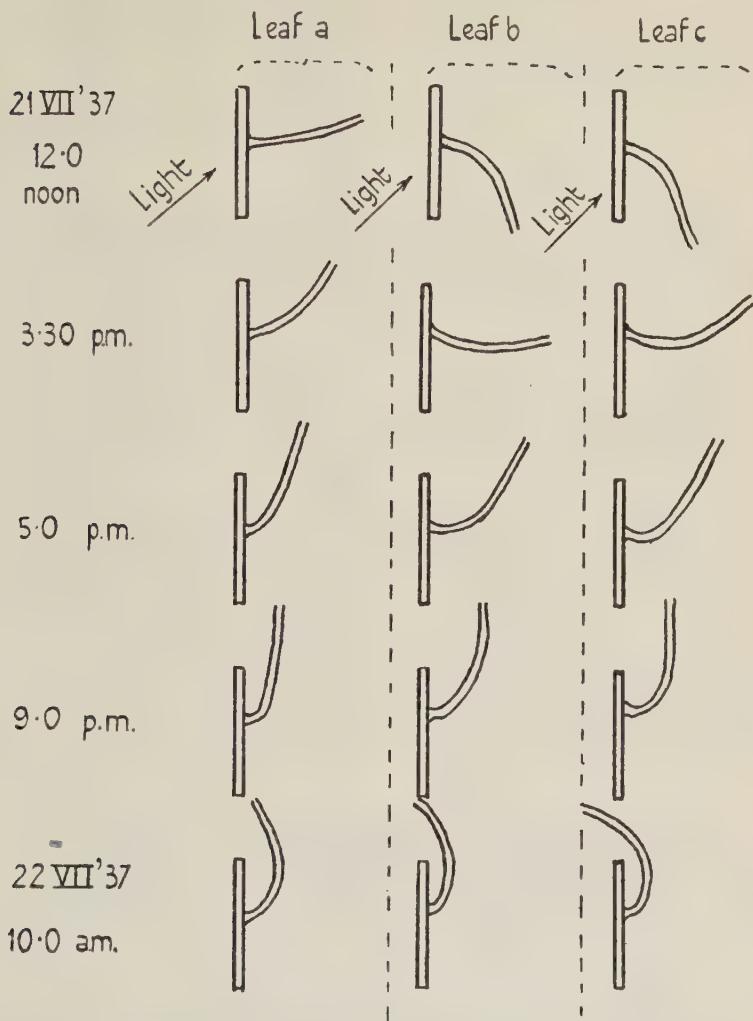


FIG. 1. Three representative leaves of *Limnanthemum peltatum* from experiment 2; laminae held in a vertical plane, light incident 45° below the horizontal. The rapid curvature of the petioles upwards indicates a high geotropic sensitivity; there is no indication of phototropic sensitivity.

Experiment 5. The reaction of the petiole under oblique illumination of the lamina was also investigated in leaves on a horizontal klinostat. A piece of rubber sheet was stretched and tied over the mouth of a broad glass bottle filled with water. A hole was made in the rubber sheet with a needle; by

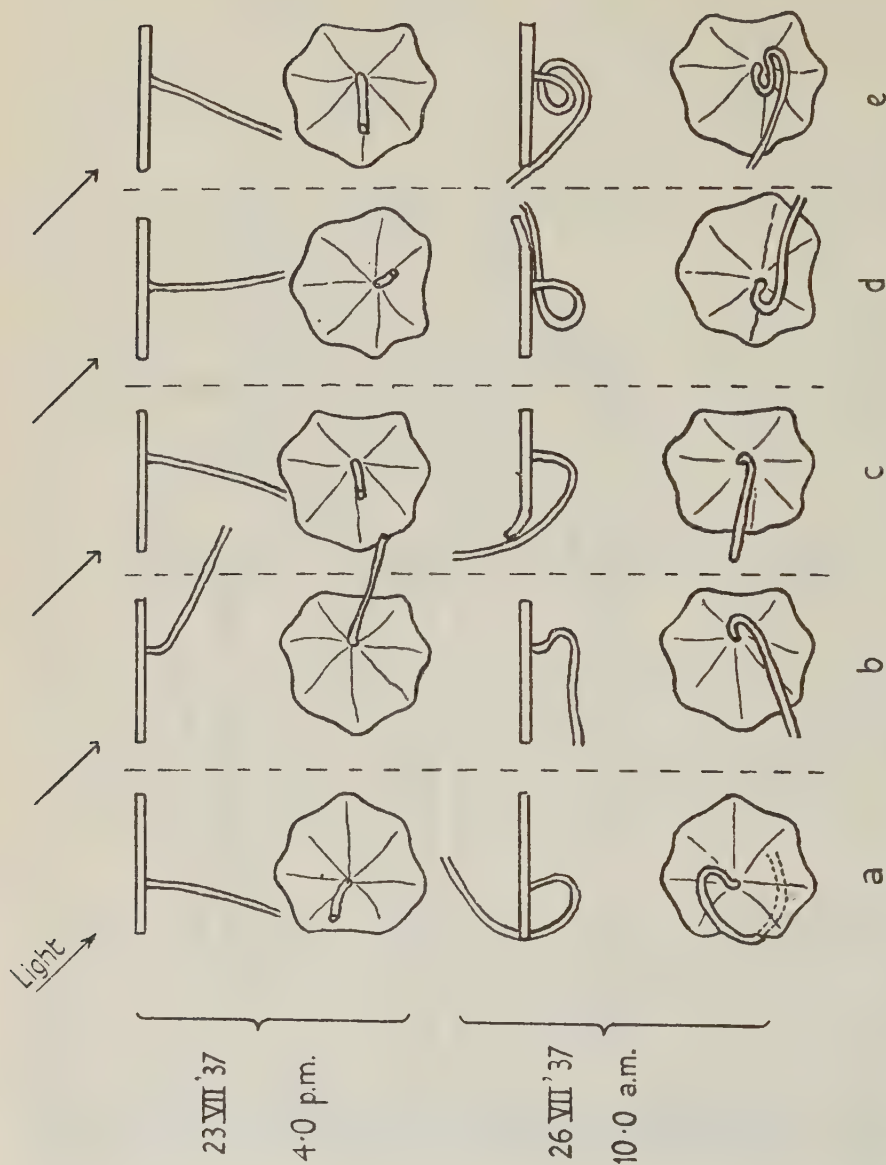


FIG. 2. Five representative leaves of *Tropaeolum majus* from experiment 4: laminae held in a horizontal plane and illuminated obliquely from above. The curvature of the petioles towards the incident light indicates marked phototropic sensitivity.

stretching the rubber it was possible to insert the petiole of the experimental leaf so that the lamina of the leaf was close against the rubber and the petiole entirely within the bottle. The bottle, with its leaf, was fixed to a horizontal klinostat with the lamina in a vertical plane. During its rotation the klinostat carried round an electric bulb which provided a source of illumination and which retained the same relative position in respect to the leaf. Thus, any resulting curvatures of the petiole could be ascribed to the effect of the unilateral illumination.

The result of such experiments was that the petioles curved towards the source of light.

Experiment 6. To determine the effect of gravity on the orientation of the leaves, detached leaves were set up in damp air in a glass tank as in experiment 2, but in complete darkness. Such experiments showed that whatever the orientation of the lamina in a vertical plane, an upward geotropic curvature of the petiole occurs in the course of a few hours.

Experiment 7. Experiments were set up similar to the foregoing with the laminae in a vertical plane, except that the leaves were illuminated by light incident at 45° below the horizontal, an exact parallel to experiment 2 with *L. peltatum*.

In the course of twenty-four hours there was in most cases a definitely upward curvature of the petioles, but much less pronounced than in complete darkness (experiment 6). In one instance a downward curvature occurred.

It may be concluded, therefore, that in *T. majus* curvature of the petiole has relation to the direction of incident light and also to the stimulus of gravity; and that when the stimuli of light and gravity are antagonistic to one another the position assumed is a resultant between the effects produced by each of them.

LOCALIZATION OF THE GRAVITY-SENSORY REGION

Experiment 8. Leaves of *L. peltatum*, from which the laminae had been almost completely removed, were set up in a damp chamber as in experiment 2, together with control leaves with the laminae intact, and placed in the dark. After twenty-four hours the petioles of leaves that had had the laminae removed showed no change of position, in contrast with those of control leaves which were all pointing vertically upwards. Evidently, removal of the lamina largely destroys the capacity of a leaf to respond to geotropic stimulus by curvature of the petiole.

Experiments with *Tropaeolum* similar to the foregoing gave like results.

LOCALIZATION OF THE LIGHT-SENSORY REGION

Experiment 9. *Tropaeolum* leaves were floated on water, with the incident light falling on them at an angle of 45° above the horizontal, as in experiment 1.

In some of these leaves the laminae were almost entirely removed, in some half removed, and in others left intact. The reactions obtained in these three cases were: (1) a marked curvature of the petioles towards the incident light in the controls with intact laminae; (2) an equally marked curvature in those in which only half of the lamina was present; (3) no appreciable curvature in those with the laminae completely removed. It may be concluded that the sensory region of the leaf in respect to light is located in the lamina, and that illumination of only half of this organ is sufficient to produce curvature of the petiole such as would orientate the lamina perpendicularly to the source of light under natural conditions.

Experiment 10. *Limnanthemum* and *Tropaeolum* leaves were chosen, having the laminae set obliquely to the petioles. The lower ends of the petioles were fixed to the bottom of museum jars full of water in such a way that each of the laminae was at an angle of 45° to a source of light in front of the jars.

The reactions occurring in the course of eighteen hours in two representative examples are shown in Fig. 3. In the case of *Limnanthemum*, the curvature of the petiole has turned the lamina into a position more nearly *horizontal*, but there has been no twist about a vertical axis to bring the lamina perpendicular to the incident light. In *Tropaeolum* the petiole has bent so as to bring the lamina into a more nearly *vertical* position and perpendicular to the incident light, the latter relation being assisted by a twisting of the petiole about a vertical axis. In *Limnanthemum* orientation of the lamina appears to be controlled solely by gravity; in *Tropaeolum* mainly by the direction of the incident light.

The final conclusions to be drawn from these experiments as a whole are that in the case of *L. peltatum* the lamina is highly sensitive to the stimulus of gravity, the reaction being such as to bring the lamina into a horizontal position by means of appropriate curvatures of the petiole. Light has little, if any, effect in orientating the leaf. In the case of *Tropaeolum*, the lamina is likewise sensitive to the stimulus of gravity, but is also sensitive to the direction of the incident light. The position assumed by the leaf, and determined by bending and twisting of the petiole, is a resultant between the geotropic and phototropic stimuli, the latter being the more effective.

It is presumed that the failure to respond to a phototropic stimulus characteristic of *L. peltatum* is a common feature of the floating leaves of water plants; it is certainly true for *Nymphaea alba*, which has been used for a series of experiments parallel to those described above. On biological grounds the advantage of this characteristic is obvious: floating leaves which responded to oblique illumination could only do so by raising one side of the leaf out of the water or by submerging one side, either of which would be unsatisfactory in view of the structure of these leaves, control of the transpiration rate being lost in the first case and gaseous exchange being interfered with in the second.

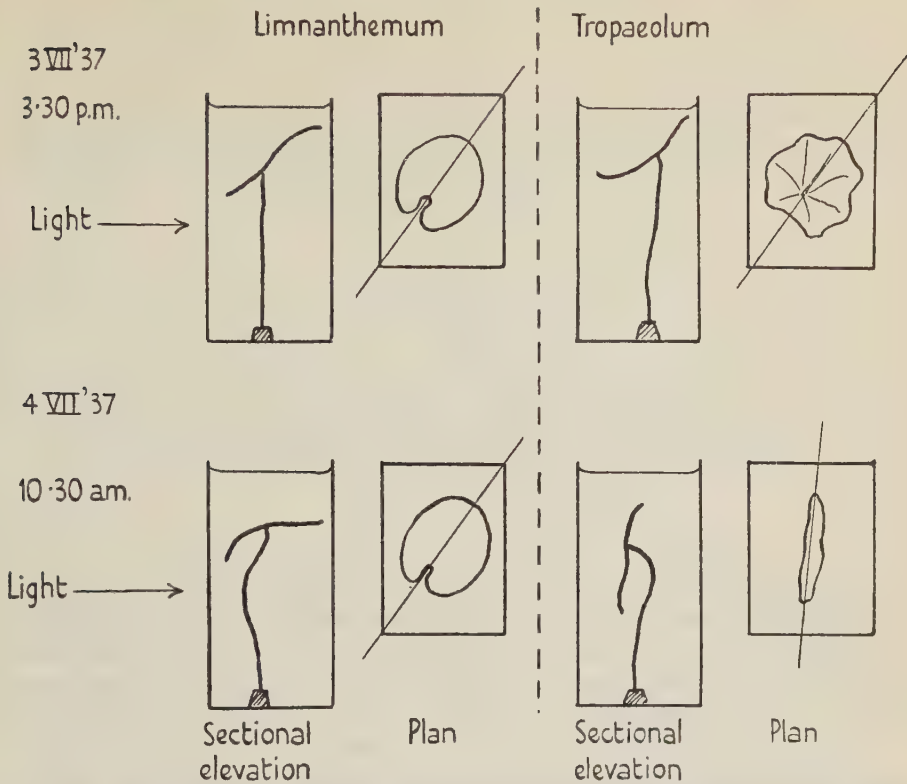


FIG. 3. Totally immersed leaves of *Limnanthemum peltatum* and *Tropaeolum majus*: base of petiole fixed to bottom of jar; lateral illumination (experiment 10). In the former there is mainly a geotropic reaction, the lamina becoming nearly horizontal; in the latter there is mainly a phototropic reaction and the lamina turns towards the vertical plane perpendicular to the incident light.

SUMMARY

Experiments are recorded which indicate that leaves of both *Limnanthemum peltatum* and *Tropaeolum majus* tend to orientate their laminae in relation to gravity by curvatures of the petioles in such a way as to bring the laminae into a horizontal plane. The leaves of *T. majus* are in addition sensitive to unilateral illumination, those of *L. peltatum* are not. On biological grounds this reaction to light may be expected as a general difference between aerial and floating leaves.

A Comparative Physiological Study of Sugar-beet and Mangold with respect to Growth and Sugar Accumulation

II. Changes in Sugar Content

BY

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With ten Figures in the Text

INTRODUCTION

AN account has been given in a previous paper (Watson and Baptiste, 1938) of the changes observed in dry weight and water content of leaf, petiole, and root, in leaf number and in leaf area, during the growth of sugar-beet and mangold sown on six successive dates in 1934. In the course of this work, samples were taken for estimation of the sugar content of all parts of the plants, and the results obtained form the subject of the present paper.

Knowles, Watkin, and Hendry (1934) have followed the changes in sucrose and reducing-sugar content of leaves and roots during the growth of sugar-beet, but they did not separate leaf lamina and petiole. Their work was mainly concerned with changes in the constituents of the ash. They found that the sucrose content of the leaf varied rather irregularly between 2.0 per cent. and 4.9 per cent. of the dry matter and concluded that it 'maintained a steady level throughout'. The reducing sugar content increased steadily from 1 per cent. in May to 11 per cent. in September. In the root, the sucrose content increased rapidly from 7 per cent. at the end of May to 33 per cent. at the end of June, then at a decreasing rate to 70 per cent. in August, and later there was evidence of a fall to 67 per cent. in September. The reducing sugar content of the root rose from 2.5 per cent. at the end of May to a maximum of 3.3 per cent. in July, subsequently falling slowly to 1.2 per cent. at the end of September.

Earlier work on the sugar content of the leaf is summarized by Davis, Daish, and Sawyer (1916), in their paper on diurnal changes. Much of it is untrustworthy because of inaccurate methods of estimation and inadequate sampling, and it includes no detailed study of ontogenetic changes. Wagner (1932) determined the sucrose content of the root at successive stages. His

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results are somewhat erratic, but show an increase from 39 per cent. at the end of June to a maximum of 69 per cent. in August followed by a fall to 61 per cent. in October. The results of investigations on the sucrose content of the root expressed as per cent. of fresh weight, made with the object of determining the time of optimum sugar yield, are summarized by Roemer (1927). They show that the highest sucrose content may occur at any time from the end of September to November, and high July temperatures apparently induce an early maximum.

Onslow (1931) has pointed out that it is desirable to express sugar contents as concentrations, that is, to express them on the basis of the water content of the tissue, as well as on the more usual dry-matter basis, for it is presumably the concentration of sugar which determines its physiological activity. This seemed particularly important in the present work, where sugar-beet and mangold are compared, for the water content of the two plants differs widely. Accordingly, the results have been expressed both as g. sugar per 100 g. dry matter, and per 100 g. water.

ARRANGEMENT OF PLOTS AND METHODS OF SAMPLING

A full description of the arrangement of plots and of the sampling procedure has already been given. The experiment consisted of six blocks sown at intervals of a fortnight, one at each sowing date beginning on April 9, 1934. Each block consisted of two plots, one sown with sugar-beet, variety Kleinwanzleben E, and the other with mangold, variety Garton's Yellow Globe. The dates of sowing were I. April 9, II. April 23, III. May 7, IV. May 21, V. June 4, and VI. June 18.

At intervals of a fortnight from thinning, samples were taken from each plot. With the exception of those taken immediately before thinning, each sample consisted of twenty plants. At each sampling time the sampling was spread over two days, the first three sowings being sampled on the first day, and the last three sowings on the second day. The order of sampling the three sowings on any day was determined at random. The first was sampled at about 10 a.m., the second at 12.30 p.m., and the third at 4 p.m. Part of the variation between sowings on any sampling occasion was, therefore, attributable to diurnal variation, and an attempt has been made to evaluate the magnitude of this.

Sub-samples consisting of about 100 g. fresh weight of leaf lamina, petiole, and root were taken from each sample of twenty plants. The petiole samples also included some stem tissue, for the separation of petiole and root was made by cutting off the top of the plant at the level of the lowest leaf. The stem tissue formed a greater proportion of the petiole fraction in sugar-beet than in mangold.

Sugar estimations were made on every sampling occasion up to time 8 except that, at time 7, sub-samples for the first sowing of sugar-beet and of mangold were accidentally omitted. After time 8, samples for analysis were

taken at monthly intervals on every second sampling occasion, but for the last three sowings analyses were also made for sampling time 9. The samples were taken less frequently in the later stages of growth, because it seemed probable that the sugar content of the plant would then be changing slowly. The samples of petiole of sowing VI of sugar-beet and mangold for sampling time 10 were accidentally mixed after extraction, so that no analyses were possible.

ANALYTICAL PROCEDURE

(a) *Extraction.*

The samples of petiole and root were cut up as rapidly as possible. The leaf lamina samples obtained by punching were found to be already sufficiently divided. Each sample was dropped into 500 ml. boiling 95 per cent. alcohol, and the boiling was continued under a reflux condenser for at least half an hour. The sample and extract were allowed to cool, and were then transferred to a quart bottle in which they were stored.

The insoluble material was filtered off on stainless steel wire gauze, well washed with 95 per cent. alcohol, dried in an electric oven at 100° C. and ground as finely as possible by hand in a covered mortar. The powder was transferred to a thimble, and extracted in a Soxhlet extractor for 8 hours, using about 300 ml. of the alcohol from the preliminary extraction. Fresh alcohol was not used, as it was essential to keep the total volume of extract at a minimum. Two or three fine glass capillaries were put into the alcohol to ensure smooth boiling, and the boiling flasks were immersed to the neck in a water-bath heated to 90–95° C., so as to give as uniform heating as possible.

After the Soxhlet extraction, the thimbles and contents were dried at 100° C., and the insoluble residue was weighed. The extract, including the residue from the preliminary extraction, was made up to 750 ml. with 95 per cent. alcohol. A 25-ml. aliquot was taken and the soluble material in this was weighed after drying at 100° C. The total dry weight of the sample was obtained by adding the weight of alcohol soluble material in the whole extract to the weight of insoluble material.

250 ml. of the extract were taken and evaporated under reduced pressure at 30–35° C. until all trace of alcohol was removed. The residue was taken up in water, and made up to 200 ml. Gummy material insoluble in water was removed by filtering through a small cotton-wool plug in the neck of a funnel. The aqueous solution was kept in a stoppered flask under a layer of toluene, and portions of it, suitably diluted, were used for the sugar estimations.

(b) *Sugar estimations.*

The sugars in the aqueous extract were estimated by means of the Harding and Downs copper reduction method as modified by Vanderplank (1936). The unclarified extract was used, and non-sugar reducing substances present

were estimated after fermentation with baker's yeast. Three estimations were made on each extract: (1) reduction before inversion; (2) reduction after inversion with invertase; (3) reduction after fermentation. The difference between (1) and (3) gave the reducing sugar content, and that between (2) and (1) the sucrose content. Glucose and fructose were not estimated separately. As fructose gives a slightly lower reduction with the Harding and Downs reagent than glucose, calibration data for invert sugar were used in calculating sugar content from the reduction values in ml. 0.01N thiosulphate. Thus the results are all expressed as g. invert sugar per 100 g. dry matter or per 100 g. water.

The procedure for inversion and fermentation was similar to that used by Vanderplank. 10 ml. of diluted extract containing approximately 20 mg. total sugar were incubated at 35° C. with 2 ml. of a 0.2 per cent. solution of invertase for 2–3 days, with toluene as a disinfectant. The solution was then made up to 50 ml. and the sugars estimated.

The fermentation was carried out in 50 ml. centrifuge tubes. 1 g. of washed yeast suspended in water was centrifuged, the supernatant water was poured away, and the sides of the tube well dried with filter paper. 20 ml. of extract diluted to contain not more than 10 mg. total sugar and free of toluene was pipetted into the tube, well stirred to disperse the yeast, and placed in an incubator at 35° C. for two hours, with occasional shaking to keep the yeast dispersed. The yeast was then spun down and the non-fermentable residual reduction was estimated. Blank estimations with distilled water instead of the plant extract were made for both inversion and fermentation, as both invertase and yeast gave a small reduction.

RESULTS

1. *Statistical analysis.*

The method of statistical analysis was the same as that already described for the dry weight and water-content data. For ease of computation the data for sampling times previous to time 5, and for sampling time 9 were omitted, because these did not include all sowings. The missing value for sowing I of sugar-beet and mangold at sampling time 7, and for the petiole of sowing VI at sampling time 10, were computed by the method given by Yates (1933).

Linear regressions on sowing date were fitted to the results for each sampling time, so that the sowings were compared at the same calendar dates. The alternative method of comparison, at equivalent times measured from sowing date, could not conveniently be made, because of the omission of sugar determinations for sampling times 9, 11, and 13. There was no indication in any of the data of curvature in the relation to sowing date, and accordingly it was considered unnecessary to fit second order terms in the regression.

The primary data are too bulky to be published in full, and all the information derived from them relevant to time changes and the effect of sowing date are presented in Figs. 1–8, in which the mean sugar content for the six sowing

dates of sugar-beet and of mangold, and the linear regression coefficients on sowing date are plotted against time. The magnitude of significant differences is shown by small vertical lines at the right-hand side of each graph.

2. *Reducing sugars.*

The mean reducing sugar content of all sowing dates, for all parts of the plants, calculated on the basis of dry weight, is plotted in Fig. 1, and on the water-content basis in Fig. 2. The effect of sowing date, estimated as a linear regression, is shown in Figs. 3 and 4.

(a) *Leaf lamina.* The reducing sugar content of the leaf lamina was consistently greater in sugar-beet than in mangold. The difference was relatively greater on the water-content basis than on the dry-matter basis, particularly in the later stages of growth.

The mean value for all sowings (Figs. 1 and 2) increased greatly with time. There is a suggestion that the increase was more rapid in the later stages, but this is mainly dependent on the low values at sampling time 10, apart from which the drift was almost linear.

At first the effect of sowing date (Figs. 3 and 4) was small and not significant, but from sampling time 10 onwards the regression coefficients were positive and increased with age. There was no significant difference at any time between the coefficients for sugar-beet and mangold. At the end of the growth period, the effect of later sowing in increasing the reducing sugar content was very considerable. In sugar-beet, at sampling time 14, the increase per week of later sowing was 0.55 g. per 100 g. dry matter, or about 5 per cent. of the mean value, 11.4, so that for the maximum range of ten weeks between sowings I and VI the increase amounted to approximately 50 per cent. of the mean.

The changes with time in mean reducing sugar content and in the effect of sowing date were very similar whether the results are expressed on the basis of dry matter or of water content.

(b) *Petiole.* The mean reducing sugar content of the petiole (Figs. 1 and 2) was greater than that of the lamina, except on the water-content basis for the mangold at times 12 and 14. It was much higher in sugar-beet than in mangold, and the difference was relatively greater on the water-content basis, owing to the lower water content of the sugar-beet petioles.

The reducing sugar content of the petiole increased with time, but the change was proportionately smaller than in the lamina. In mangold the increase was almost linear, but in sugar-beet, particularly on the basis of dry weight, the rate of increase was less in the later stages of growth and there was actually a slight, but not significant, fall between times 12 and 14.

The effect of sowing date varied widely with sampling time. The linear regression coefficient on sowing date (Figs. 3 and 4) increased rapidly from large negative values in the early samplings through zero to positive values, and later there was some evidence of a fall, particularly in mangold. The

depression of reducing sugar content by later sowing in the early stages of growth was greater in sugar-beet than in mangold, but the change to a positive effect was more prolonged so that in the later stages the effect of sowing date was almost the same for sugar-beet and mangold.

Expressing the reducing sugar content on the basis of water content

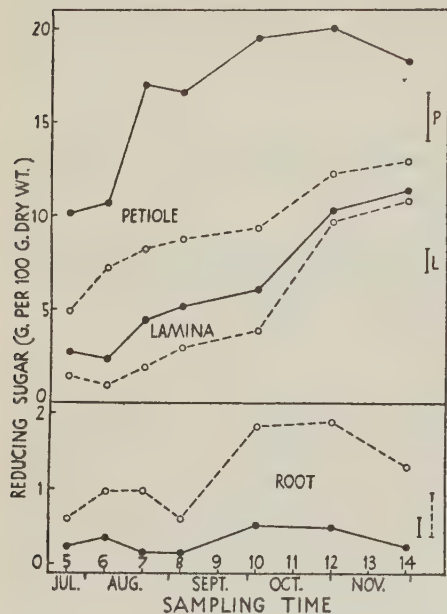


FIG. 1.

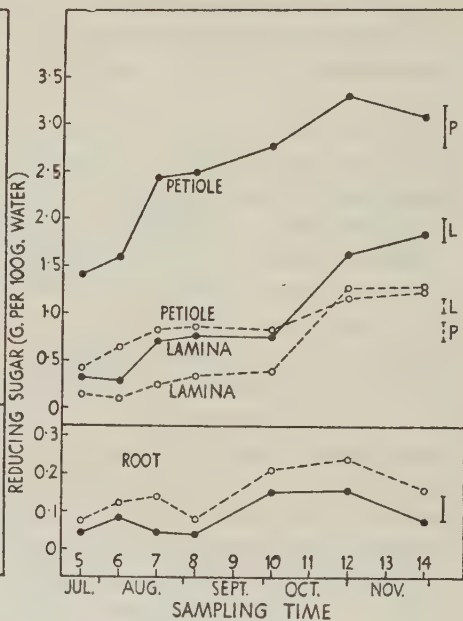


FIG. 2.

FIGS. 1 and 2. Fig. 1. Change with time of reducing sugars expressed as gm./100 gm. dry matter, mean of all sowings. Black circles, sugar-beet; open circles, mangold. Short vertical lines represent significant differences for petiole and lamina in both sugar-beet and mangold. In the lower figure separate values are given for the two plants. Fig. 2. Change with time of reducing sugars expressed as gm./100 gm. water, mean of all sowings. Black circles, sugar-beet; open circles, mangold. Short vertical lines represent significant differences for petiole and lamina in both sugar-beet and mangold. In the upper figure separate values are given for the two plants.

emphasized the difference between mangold and sugar-beet. The regression coefficient on sowing date continued to have negative values for a longer period, especially in sugar-beet, and the positive values attained at the end of the growth period were relatively smaller than when the dry-weight basis was used.

(c) *Root*. The reducing sugar content of the root of mangold was higher than that of sugar-beet, but it was low in both plants. In sugar-beet its highest value for the mean of all sowings (Fig. 1) was 0.5 per cent. of the dry weight, as compared with 20 per cent. in the petiole and 11 per cent. in the leaf lamina. On the basis of water content (Fig. 2), the difference between sugar-beet and mangold was smaller but still significant. The variation of

mean reducing sugar content with time was somewhat irregular, but there was clearly an increase in the later stages of growth. It was small both in absolute magnitude and relative to the mean value, compared with the corresponding increases in lamina and petiole.

The regression coefficients on sowing date (Figs. 3 and 4) did not differ

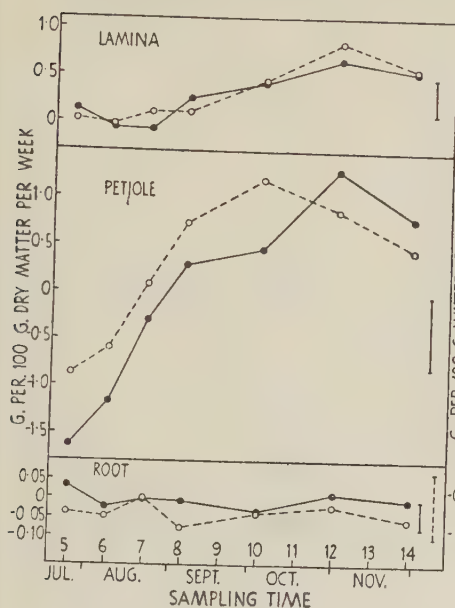


FIG. 3.

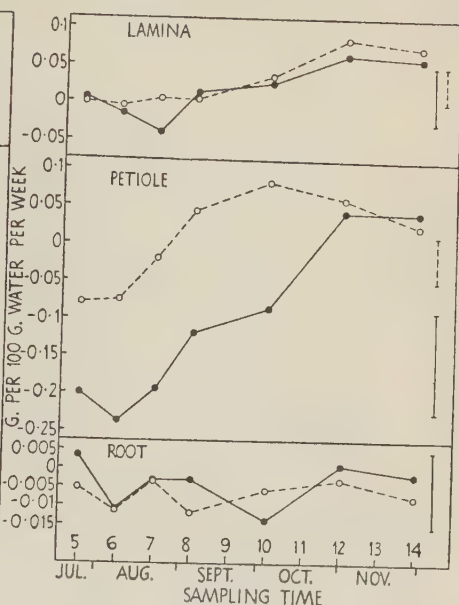


FIG. 4.

FIGS. 3 and 4. Fig. 3. Linear regression coefficients on sowing date for reducing sugars expressed as gm./100 gm. dry matter. Black circles, sugar-beet; open circles, mangold. Short vertical lines represent significant differences. Fig. 4. Linear regression coefficients on sowing date for reducing sugars expressed as gm./100 gm. water. Black circles, sugar-beet; open circles, mangold. Short vertical lines represent significant differences.

for sugar-beet and mangold and the variation with time was irregular and not significant. The mean regression coefficients of all sampling times were negative, significantly so when the sugar content was calculated on the water-content basis. Thus the only clear effect of later sowing on the reducing sugar content of the root was that on the average of all sampling times later sowing caused a small depression.

3. Sucrose.

Fig. 5 shows the variation with time in the mean sucrose content of all sowings for all parts of the plant of sugar-beet and mangold, calculated on the basis of dry weight, and Fig. 6 the corresponding changes on a water-content basis. The linear regression coefficients of sucrose content on sowing date are plotted in Figs. 7 and 8.

(a) *Leaf lamina.* The mean sucrose content of all sowings expressed as

per cent. of dry matter (Fig. 5) was almost the same for sugar-beet and mangold, but on the water-content basis (Fig. 6) it was always slightly greater in sugar-beet. It increased steadily until sampling time 12, but there was a sharp fall at sampling time 14. The increase with time was not so great as

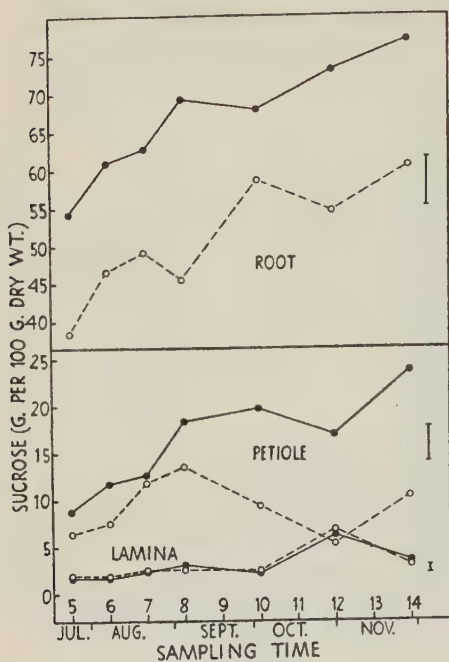


FIG. 5.

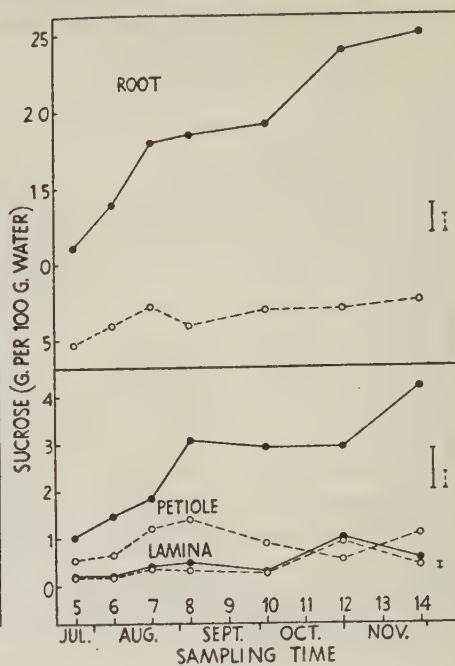


FIG. 6.

FIGS. 5 and 6. Fig. 5. Change of sucrose content with time expressed as gm./100 gm. dry matter. Black and open circles and vertical lines as in earlier figures. Fig. 6. Change of sucrose content with time expressed as gm./100 gm. water. Black and open circles and vertical lines as in earlier figures.

that found for the reducing sugar content, so that although sucrose and reducing sugar were present in roughly equal amounts at sampling time 5, in the later stages of growth reducing sugar greatly exceeded sucrose.

The linear regression coefficients on sowing date (Figs. 7 and 8) showed a drift with time from negative to positive values similar to that found for reducing sugars. The positive values finally attained were smaller, both absolutely and relative to the mean sugar content, than those of reducing sugars. Although the difference between the coefficients for sugar-beet and mangold was never significant, the values for sugar-beet always lay below those for mangold, that is to say, the depression with later sowing in the early stages was greater, and the increase in the later stages smaller, than in mangold. On the water-content basis, the regression coefficient for sugar-beet did not in fact rise above zero.

(b) *Petiole.* The mean sucrose content of the petiole (Figs. 5 and 6) was

higher than that of the lamina, except for mangold at sampling time 10. It was greater in sugar-beet than in mangold, and because of the higher petiole water content of mangold the difference was greater when the results were expressed on the water-content basis.

In sugar-beet, the sucrose content increased with time, and the rate of

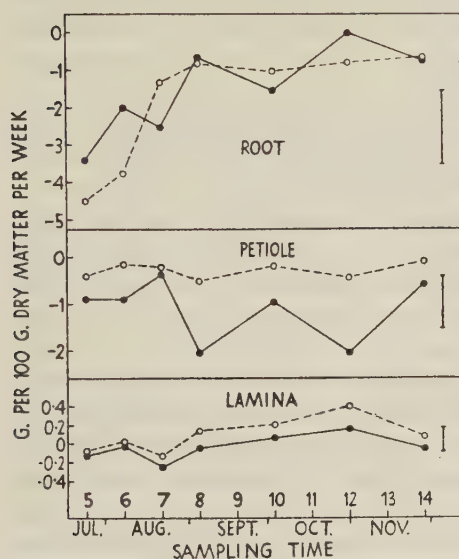


FIG. 7.

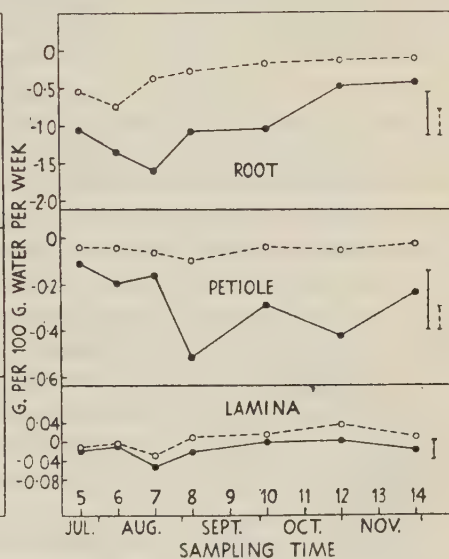


FIG. 8.

FIGS. 7 and 8. Fig. 7. Linear regression coefficients on sowing date for sucrose expressed as gm./100 gm. dry matter. Black and open circles and vertical lines as in earlier figures. Fig. 8. Linear regression coefficients on sowing date for sucrose expressed as gm./100 gm. water. Black and open circles and vertical lines as in earlier figures.

increase became less rapid with advancing age. In mangold there was a similar increase of sucrose content in the early stages, but a maximum was reached at sampling time 8, after which there was a sharp fall to time 12, followed by a rise in the last samples.

The regression coefficients on sowing date (Figs. 7 and 8) were always negative, indicating a depression with later sowing. The depression was much greater in sugar-beet than in mangold. The variation of the regression coefficients with time was irregular, but there is some indication that the depression due to later sowing was greater in the middle of the growth period than at the beginning and end.

(c) *Root.* The mean sucrose content of the root for all sowings (Figs. 5 and 6) increased throughout the experimental period. The rate of increase declined with age, but the rise of sucrose content was still continuing at the last sampling time, although the difference between the values at times 12 and 14 was not significant. In the very early stages of growth, immediately after thinning, the sucrose content increased very rapidly. This phase is

obscured by the use of the mean of all sowing dates to indicate the time changes. The sucrose content of the root at the time of thinning was variable: the different sowings were not thinned at exactly the same stage of development. The lowest sucrose content recorded in the sugar-beet root was 13 per cent. of the dry matter, and this increased to 30 per cent. a fortnight after thinning and to over 40 per cent. after four weeks. The corresponding figures in mangold were 4 per cent., 8 per cent., and 20 per cent. Thus even very young roots had a high sucrose content, and it is not possible to distinguish a phase of growth followed by a phase of storage of sucrose. The high sucrose content appears to be an inherent property of the organization of the root cells, rather than the result of a temporary accumulation of carbohydrate in excess of immediate requirements, which may be utilized in growth in the flowering year. This is supported by the observations of Wagner (1932) who found that only a small fraction, roughly one-third, of the sucrose in the root of sugar-beet was removed in the second year of growth, so that from the initial value of 65 per cent. in June, the sucrose content of the root fell in September to only 50 per cent. of the dry weight.

The sucrose content of the root of sugar-beet always greatly exceeded that of mangold, and on the dry-weight basis, the means of all sowings showed an almost constant difference of 15 per cent. The difference was relatively much greater on the water-content basis, and it increased steadily with advancing age.

The linear regression coefficients on sowing date (Figs. 7 and 8) were consistently negative, that is to say, the sucrose content of the root was always depressed by later sowing. The depression was very large in the early samplings, subsequently decreasing, at first rapidly and later more slowly, so that at the end of the growth period the regression coefficients assumed steady values. On the basis of dry weight, the regression coefficients were almost the same for sugar-beet and mangold, but on the basis of water content, those of sugar-beet had greater negative values. The change with time of the regression coefficients was more rapid on the dry-weight basis than on the water-content basis, and the steady value was attained earlier.

4. *Diurnal Variation of Sugar Content.*

The sugar-content data were examined for evidence of diurnal variation by the method previously used (Watson and Baptiste, 1938) for water content. A regression equation of the form

$$(y - \bar{y}) = b_1(x_1 - \bar{x}_1) + b_2(x_2 - \bar{x}_2)$$

was fitted to the results for each sampling time, where y is the sugar content, x_1 the time of sowing in weeks from the first sowing date, and x_2 the time of day of sampling, expressed as the order of sampling, representing the first sampling on any day as -1 , the second as 0 , and the third as $+1$. The method is obviously not capable of detecting small changes because of the

high variability between plants. No significant differences in b_2 , the regression coefficient on time of day, were found between sugar-beet and mangold or between sampling times, so that only the mean regressions for both crops and all sampling times need be considered. The mean regression coefficients are shown with their standard errors in Table I. The sowing date regression coefficients (b_1) of the regression equations are omitted, as they differed little from the values obtained when the regression was fitted on sowing date alone.

TABLE I

*Linear Regression Coefficients of Sugar Content on Time of Day of Sampling.
(Mean of Sugar-beet and Mangold and of All Sampling Times)*

		Lamina	Petiole	Root
Sucrose	per 100 g.	0.309 ± 0.085	0.310 ± 0.303	-0.146 ± 0.663
Reducing sugar	dry matter.	0.234 ± 0.172	0.240 ± 0.392	-0.007 ± 0.052

When the sugar content was expressed as per cent. of dry matter, the regression coefficient for sucrose content of the lamina was the only one to reach the level of significance. In accordance with expectation it was positive, showing that the mean increase in sucrose content (2×0.309) between the first and last sampling of a day (10 a.m. to 4 p.m.) was about 21 per cent. of the mean sucrose content (2.95 g. per 100 g. dry matter). The regression coefficient for reducing sugar in the lamina slightly exceeded its standard error but was far from significance. It was not as great as the sucrose coefficient. Thus the data show an increase in the sucrose content of the lamina during the day, but no other clear effects. The remarkable similarity between the mean regression coefficients for lamina and petiole was possibly fortuitous, for no evidence of such a similarity was found in the regression coefficients for individual sampling times.

Davis, Daish, and Sawyer (1916) have studied the variation in sugar content as per cent. of dry matter of mangold leaves during three twenty-four hour periods. The changes observed in the interval from 10 a.m. to 4 p.m. varied widely. In the first experiment, reducing sugars decreased greatly and sucrose remained constant; in the second, reducing sugars increased and sucrose decreased, and in the third, reducing sugars did not change, while sucrose showed a large increase. In the results of the present experiment no such inconsistency was found. The regression coefficients for individual sampling times, though not significant, were all positive for sucrose, and for reducing sugars all were positive except that for sampling time 8.

The regression coefficients of sucrose and reducing sugar per 100 g. of water on time of day were also calculated for the lamina, and both were found to be positive and significant. This increase during the day in the concentration of sugars in the leaf was, however, partly the result of the diurnal fall of water content already demonstrated (Watson and Baptiste, 1938) and is not necessarily evidence of an increase in the amount of sugar in the leaf.

5. Starch.

Qualitative tests for the presence of starch were made on the insoluble residues after alcohol extraction. A portion of the residue was heated on a boiling water-bath with saturated calcium chloride solution, and the extract was tested for starch by adding iodine solution. Starch was found in all the samples of leaf lamina examined, both of sugar-beet and mangold up to

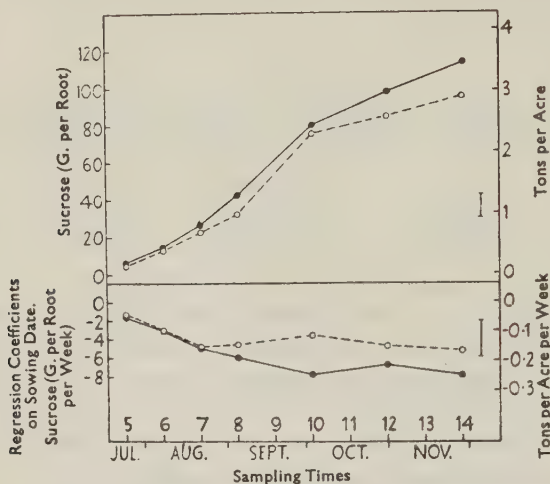


FIG. 9. Upper figure shows change with time of yield of sucrose as gm. per root and tons per acre. Lower figure gives regression coefficients on sowing date for sucrose expressed as gm. per plant per week. Black and open circles and vertical lines as in earlier figures.

sampling time 9, and a trace was present in some of the samples of time 10. Starch was also present in the early samples of petiole, apparently in smaller amounts than in the lamina, but none was detected in samples later than those of time 5. None of the root samples gave a positive test. These results conflict with those of Davis, Daish, and Sawyer (1916) on mangold, who found starch only in the lamina of very young plants.

6. Yield of sucrose per root and per acre.

From an agricultural point of view the yield of sucrose per root or per unit area of crop is the most important growth function. The mean weight of sucrose per root for all sowings, and the regression coefficients on sowing date are shown in Fig. 9, where a scale of tons per acre is also given.

The mean weight of sucrose per root increased throughout growth, and the rate of increase was more rapid in the middle of the growth period than at the beginning and end, so that the curve had an S-shaped form similar to that of dry weight. The trend of the graphs indicates that the increase was still proceeding at the last sampling time. The increase between sampling times 12 and 14 was significant for sugar-beet, but not quite significant for mangold. The weight of sucrose per acre was consistently greater in sugar-

beet than in mangold, but the difference was not great, because the greater sucrose content of sugar-beet was offset by the greater root weight of mangold. The final yield of sucrose, over 3 tons per acre in sugar-beet, was high, and

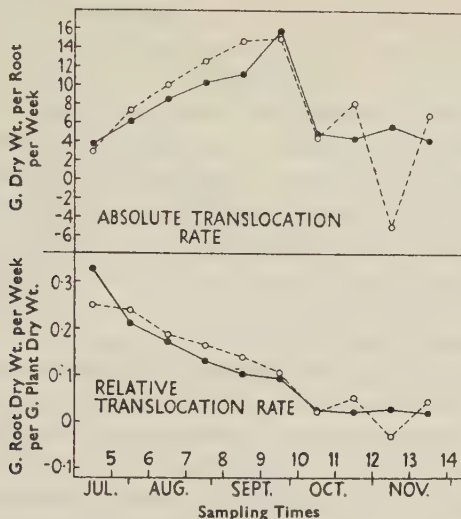


FIG. 10. Translocation rate, mean of all sowings. The upper graph shows the rate of increase in dry weight of the root, and the lower graph shows the rate of increase in dry weight of the root per unit dry weight of the whole plant. Black circles, sugar-beet; open circles, mangold.

this may partly be attributed to the rejection in sampling of abnormally spaced plants.

The regression coefficients on sowing date (Fig. 9) were negative at all times, showing that later sowing depressed the weight of sucrose per root. The depression at first increased with time; later, from sampling time 10 onwards, reaching an almost steady value which was greater in sugar-beet than in mangold. The depression of final sucrose yield, amounting to 0.24 tons per acre per week of later sowing, represents a large reduction in the agricultural value of the sugar-beet crop. Inspection of the yields of sucrose for individual sowings (Table II) suggests that the decline of yield with later sowing was less rapid in the early sowings than the late.

TABLE II

Total Sucrose in Root at Sampling Time 14. Tons per acre

Sowing date	I	II	III	IV	V	VI
Sugar-beet	4.7	3.8	4.0	3.7	2.6	2.0
Mangold	3.5	3.6	3.2	2.6	2.6	1.9

This is a reflection of a similar effect in the yield of dry matter, for there was no indication of any curvature in the graph of the relationship of sucrose content (as per cent. of dry matter) to sowing date.

DISCUSSION

In general, the content of both sucrose and reducing sugars was greater in sugar-beet than mangold. The only exceptions were that in the root, the reducing sugar content was greater in the mangold, and in the lamina, the sucrose content as per cent. of dry matter was almost identical in the two plants, though on the water-content basis it was slightly greater in sugar-beet.

The sucrose content of the root was very much greater than that of the petiole, which in turn exceeded that of the lamina. The only deviation from this steady increase in the direction from lamina to root was at sampling time 12, when the sucrose content per 100 g. water of the mangold lamina slightly exceeded that of the petiole. Reducing sugars were present in the highest concentration in the petiole, and the content of the lamina was greater than that of the root.

The sugar content of all parts of the plant increased with time, and in most cases the increase continued throughout the growth period. The sucrose content of mangold petiole was exceptional in that after sampling time 8 it fell sharply until sampling time 12, subsequently rising again. The sucrose content of the lamina and the reducing sugar content of the root of both plants, and of the petiole of sugar-beet decreased between sampling times 12 and 14. In the lamina and root the decrease was significant.

No attempt has been made to correlate with meteorological factors the deviations of sugar content from smooth trends, for the observations were made too infrequently to give information on weather effects. An examination of the data was made to determine whether correlations existed between the deviations of sucrose and reducing sugars in the same and in different parts of the plant. The graphs suggest, for example, that after the elimination of trend, the sucrose content of the lamina was correlated positively with the reducing sugar content of the lamina, and negatively with the sucrose content of the petiole, but no significant correlations were found.

The relation of sucrose to reducing sugars in the different parts of the plant is best seen from an examination of their ratio, the values of which, for the mean of all sowings, are given in Table III.

The ratio of sucrose to reducing sugar was slightly greater in mangold than in sugar-beet, except in the root. As the reducing sugar content of the lamina increased more rapidly with time than the sucrose content, the ratio tended to fall to lower values in the later samples. For sugar-beet, the ratio was always less than unity, but in mangold at the earlier sampling times the sucrose content was slightly greater than the reducing sugar content.

The ratio for the petiole was usually greater than that for the lamina, especially in sugar-beet, and the drift with time was in the opposite direction to that found in the lamina, showing with time a relatively greater increase in sucrose than in reducing sugar. The time drift was not very regular; in mangold the values for the last three sampling times were low. In the root

the ratio was very high and much higher in sugar-beet than in mangold. No regular time drift is apparent.

Knowles, Watkin, and Hendry (1934) found in the same variety of sugar-beet, grown in 1933 on a similar soil and with similar manuring except for the addition of 9 tons of dung per acre, values for the reducing sugar content

TABLE III

Ratio of Sucrose to Reducing Sugar (Mean of all Sowing Dates)

Sampling time	5	6	7	8	10	12	14
Lamina { Sugar-beet	0.62	0.69	0.55	0.61	0.37	0.61	0.30
{ Mangold	1.36	2.00	1.39	0.88	0.65	0.71	0.28
Petiole { Sugar-beet	0.72	0.92	0.75	1.23	1.05	0.88	1.36
{ Mangold	1.29	1.02	1.46	1.63	1.06	0.45	0.80
Root { Sugar-beet	265	171	408	474	127	155	342
{ Mangold	63	49	52	76	33	29	48

of the root which on the average of all samples are more than four times those given in this paper. It is not possible to decide whether this difference is a real seasonal effect, or the result of differences of analytical procedure. Vanderplank (1936) has shown that the method of clarification with basic lead acetate used by Knowles *et al.* may be inefficient, giving estimates of reducing sugars which are too high. If, however, the whole reduction by non-sugar substances (estimated in our work by the residual reduction after fermentation) is included in the estimate of reducing sugars, the values obtained are still well below those of Knowles *et al.* It is possible that their procedure of mincing the root finely before extraction caused a partial hydrolysis of sucrose, but against this it must be noted that according to Robertson, Irvine, and Dobson (1909) invertase is not present in the root of sugar-beet.

It is of interest to consider whether the data throw any light upon the mechanism of translocation of carbohydrate from leaf to root. Davis, Daish, and Sawyer (1916) concluded from their study of diurnal changes in sugar content of leaf and petiole of mangold that sucrose was formed directly by photosynthesis in the leaf mesophyll, and that it was hydrolysed to hexose, probably in the veins, and translocated to the root in this form. Their evidence for this was, firstly, that the ratio of sucrose to hexose decreased in the direction from leaf lamina to midrib to petiole. Secondly, while hexose and sucrose in the leaf showed similar diurnal variations, in the petiole the sucrose content remained almost constant throughout the day and hexose varied widely. Curtis (1935) has pointed out that neither of these observations is proof that hexose is the sugar of transport, for 'it is conceivable that the sugar was actually being transported as sucrose along a positive diffusion gradient, and the variations in hexoses merely indicate temporary accumulation and depletion in non-conducting tissues'. Nor were the observations of Davis, Daish, and Sawyer confirmed in the present work. Table III shows that the ratio of sucrose to reducing sugars was usually greater in the petiole than in the lamina. The petiole fraction here includes some stem tissue, but

in mangold this is a small proportion of the whole, and it seems unlikely that it would have a large effect on the ratio of sucrose to hexose. The regression coefficients of sugar content on time of day (Table I, p. 851) suggest that the increases of sucrose and reducing sugar contents between 10 a.m. and 4 p.m. were almost equal in lamina and petiole, and there is no indication that in the petiole reducing sugars were more variable than sucrose.

Davis, Daish, and Sawyer also observed that the ratio of sucrose to reducing sugars in the leaf declined with age. Table III shows that this was confirmed both for mangold and sugar-beet. They interpret this drift as being correlated with a change of the plant from a growing to a storage phase. Thus they state that 'during the early stages of growth . . . leaf production is the principal function of the plant and the roots are merely small tap roots' while 'in September and October . . . the call upon the cane-sugar in the leaf is actually greatest for purposes of storage in the root'. The predominance of hexoses in the leaf in the later stages of growth is taken as evidence that carbohydrate is translocated as hexose. It has already been pointed out that there is no clear distinction between phases of growth and of storage in the root, for the young roots have already a high sucrose content, so that growth and sucrose storage proceed together. Fig. 10 shows that the absolute rate of translocation, measured as the rate of increase in dry weight of the root, was greatest in August and September, falling rapidly later, while the rate of translocation relative to the total dry weight of the plant declined throughout growth. It should be noticed also that, in the argument from the difference between the ratios of sucrose to reducing sugar for leaf lamina and petiole, a relatively low hexose content of the lamina is taken as indicating translocation of hexose, while in the argument from the drift with age of the ratio of sucrose to reducing sugar in the lamina the same conclusion is deduced from an accumulation of hexose in the lamina. Thus the two arguments are mutually contradictory.

The work of Mason and Maskell (1928) on translocation in the cotton plant strongly supports the conclusion that carbohydrate is translocated in the phloem as sucrose, and that the rate and direction of movement are determined by concentration gradients in a manner analogous to diffusion. It has been shown that analysis of the whole tissue of leaf lamina, petiole, and root gave no indication of gradients of sugar concentration declining in the direction of translocation from leaf to root. The gradient of sucrose concentration ran in the opposite direction, while the concentration of reducing sugars was greatest in the petiole. Nevertheless, gradients of sugar concentration in the direction of translocation from leaf to root may exist in the phloem, which are masked in mass analyses by the differing sugar concentrations in the surrounding tissues. Curtis (1935) points out that 'mass analyses of general stem or leaf tissue may give an entirely wrong impression as to which of the constituents found are actually being transported. The seeming gradient for sucrose and for hexoses may not actually obtain in the conducting elements themselves'. In view of this it seems unprofitable to look for correlations in

our data between sugar concentration and translocation rate. A formal examination of the data was made, but no such correlations were found.

In growth studies based on measurements of change in dry weight, the choice of an appropriate method of expressing translocation rate is difficult. The net rate of translocation, measured as the rate of increase in dry weight of the root over a long period of time, must be dependent on the size of the plant, so that for the comparison of translocation rates in different periods it is necessary to eliminate plant size by the use of a relative rate of translocation. It is doubtful whether such relative rates should be expressed on the basis of mean dry weight of the whole plant, of the root or of the leaves, or on a leaf-area basis, for there seem to be no *a priori* grounds for determining whether the absolute translocation rate is more closely dependent on the size of the leaves, of the root, or of the whole plant. The ratio of increase of dry matter of the root to increase of dry matter of the whole plant, which measures the fraction of total assimilate translocated to the root, might be a more useful measure of translocation, for it eliminates the direct effect of varying assimilation rate, but at times when the dry weight of the plant is changing slowly, both numerator and denominator of this ratio are subject to proportionately large errors, and the ratio is very inaccurately determined.

According to Roemer (1927), the more numerous the rings of vascular bundles in the root of sugar-beet and the closer they lie together, the higher is the sucrose content. Similarly it is possible that the differences in sucrose content between lamina, petiole, and root are mainly determined by the relative bulk of vascular and other tissues. This would imply that throughout the plant the vascular tissue is much richer in sucrose than the surrounding parenchyma.

No effect of sowing date on the reducing sugar content of the leaf lamina could be detected in the early samples, but afterwards there appeared an increase with later sowing which became greater as growth proceeded. The sucrose content showed a similar time-trend in its relation to sowing date, but the change with time was not so great. Initially the sucrose content was depressed by later sowing, and the positive effect of later sowing towards the end of the growth period was small, particularly in sugar-beet. In the petiole, later sowing at first depressed the reducing sugar content, but this depression rapidly disappeared, and towards the end of the growth period a fairly steady positive relation to sowing date was established. The effect on sucrose content was negative throughout, the greatest depression occurring at the intermediate sampling times. The reducing sugar content of the root was slightly lower in the later sowings and the effect did not vary with the time of sampling. The sucrose content was consistently depressed by later sowing. The magnitude of the depression decreased with time so that at the end of the growth period the effect was small.

It has been shown (Watson and Baptiste, 1938) that later sowing caused an increase in area and dry weight of the leaves in the later stages of growth. The leaves of the later sowings were not only larger but also richer in sugar,

particularly in reducing sugar. On the other hand the dry weight of the root decreased with later sowing, and this was accompanied by a decrease in sucrose content. It seems probable therefore, that the effect of later sowing on leaf growth was induced by a restriction of the movement of assimilate out of the leaves, rather than by an increase in the ability of the leaves to utilize assimilate in growth, for if the latter explanation were true a reduction in sugar content in the leaf might be expected.

The effect of later sowing in reducing the total sugar yield in the root has been shown previously in experiments described by Cooke (1900) and Roemer (1927). It has also been confirmed in field experiments at Rothamsted and Woburn, the results of which are given in Table IV.

TABLE IV
Field Experiments at Rothamsted and Woburn. Sucrose as Percentage of Fresh Weight of Root, and as Yield per Acre

1935.					
Rothamsted.			Woburn.		
Date of sowing.	Per cent.	Cwt. per acre.	Date of sowing.	Per cent.	Cwt. per acre.
March 15 .	16.50	38.1	April 18 .	15.73	41.3
April 18 .	16.58	36.8	May 9 .	15.81	40.7
May 16 .	16.58	35.1	May 25 .	16.22	38.2
1936.					
April 23 .	17.72	40.6	April 8 .	17.74	54.2
May 8 .	17.70	35.9	April 27 .	17.60	48.2
May 26 .	17.54	33.3	May 15 .	17.28	38.3

In all four experiments the yield of sugar fell steadily with later sowing. The relation of sugar as percentage of fresh weight of the root to sowing date was variable. In 1935 at Rothamsted the percentage of sugar was not affected by later sowing, while at Woburn there was evidence of a small increase. In 1936, both experiments showed a decrease with later sowing. In the 1934 experiment described in this paper, later sowing depressed the sugar content of the root as percentage of dry matter, and as the water content of the root was increased by later sowing, the sugar content as percentage of fresh weight was also depressed.

SUMMARY

An account is given of the changes during growth in the sucrose and reducing sugar content (expressed per 100 g. of dry matter and per 100 g. of water) of the lamina, petiole, and root of sugar-beet and mangold sown on six occasions in 1934.

Sugar-beet had a higher content of both sucrose and reducing sugars than mangold, except that the sucrose content of the lamina was almost the same in the two plants, and in the root the reducing sugar content was greater in mangold. In general, both the sucrose and reducing sugar content of all parts of the plant increased steadily with time.

The sucrose content increased through the plant in the direction from lamina to root. The reducing sugar content was highest in the petiole, and was greater in the lamina than in the root. It is pointed out that this does not necessarily imply that translocation takes place against a gradient of sugar concentration, for gradients falling in the direction of movement may exist in the conducting tissues, which are masked in the mass analyses of lamina, petiole, and root. The data give little direct evidence on the mechanism of translocation, but they serve to illustrate some fallacies in the arguments of Davis, Daish, and Sawyer for the view that sucrose in the leaf is an immediate product of photosynthesis and that carbohydrate is translocated as hexose.

There is no clear distinction in the root between a phase of growth and a phase of sucrose storage, for the very young roots have a high sucrose content. Growth and accumulation of sucrose proceed together.

On the mean of all sampling times, a significant increase of sucrose content was found in the leaf lamina, between 10 a.m. and 4 p.m. The corresponding increase in reducing sugar was smaller and not significant. The average changes during the day in the sugar content of the petiole were almost the same as those of the leaf lamina, but were not significant. There was no indication of any diurnal variation in the root.

Later sowing caused an increase in the reducing sugar content and, to a less extent, in the sucrose content of the leaf lamina, in the later stages of growth. The reducing sugar content of the petiole was similarly affected, but the sucrose content of petiole and root was always depressed by later sowing. The reducing sugar content of the root was also slightly decreased. These results suggest that the effect of later sowing, previously demonstrated, in increasing the size and weight of the leaves, was caused by a restriction of the movement of carbohydrate out of the leaf, rather than by an increased ability of the leaf to utilize assimilate in growth.

Later sowing depressed the total yield per acre of sucrose in the root.

Copies of the primary data have been deposited in the Natural History Museum, South Kensington, and in the Library at Rothamsted Experimental Station.

The authors wish to record their indebtedness to Dr. E. C. D. Baptiste who carried out some of the sugar estimations, and to Mr. S. A. W. French for assistance in the statistical computation.

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A Study of the Polyphenol Oxidase System in Potato Tubers

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With six Figures in the Text

THERE exists a considerable body of literature concerning the reactions of the polyphenol oxidase system extracted from the tissues of certain plants and animals. From the botanical point of view the major part of the work has been done on genera in the angiospermic orders; in addition a certain number of the fungi have been tested for the presence of this system, described by Onslow (1921) as the 'direct oxidase' system and identified by the production of a blue colour when tincture of guaiacum is added to the surface of the tissue. This worker found that of 64 per cent. of the monocotyledon orders 79 per cent., and of the 60 per cent. of the dicotyledon orders 60 per cent., gave a positive reaction. Szent-Györgyi (1927) has re-examined a number of tissues and concluded that all those tested contained a phenol oxidase, and that failure to give a positive response was due to one of two causes: either the tissue lacked a catechol derivative, and therefore the *o*-quinone which oxidizes the guaiacum to give the blue colour cannot be formed, or the tissue contained a phenol oxidase, oxidizing a phenol to a quinone, which is not capable of oxidizing the guaiacum so that again the blue colour fails to develop. In the former case the addition of catechol results in the formation of a blue colour, while in the latter case the phenol oxidase appears to be incapable of oxidizing catechol to *o*-quinone and therefore, even in the presence of this substrate, a blue colour is not developed on addition of the tincture of guaiacum. He considers that the phenol oxidase system is much more widely spread in the plant kingdom than the results of Onslow would indicate. Wheldale (1911) observed a close correlation between those tissues giving a positive reaction with guaiacum and those turning brown on injury. Szent-Györgyi (1925) showed that this browning is due to the formation of a substance he calls tyrin which is not produced by direct oxidation by oxygen of the air but through the oxidative activity of the *o*-quinone. Further that the tyrin precursor and the guaiacum both require a powerful agent for their oxidation and that it is only in those tissues

which produce an *o*-quinone, having a high oxidation potential, that these reactions can occur; hence the correlation between the blueing of guaiacum and browning on injury. The exact mechanism involved in the phenol oxidase system in plant tissues has long been in dispute. Onslow and others held that two enzymes were involved, whereas Szent-Györgyi held that only one oxidizing enzyme was concerned. The evidence in support of the latter view may now be regarded as conclusive. The system therefore consists of three parts, a phenol oxidase, an aromatic hydrogen transporter, and a dehydrase. In the potato tuber the oxidase is capable of oxidizing catechol. The aromatic compound naturally present is probably oxidized to a diketoquinone (Willstätter and Müller, 1908), the quinone returning to a dihydroxy compound under the influence of the dehydrase system, the guaiacum being substituted for this in the test for the presence of the oxidase enzyme. The diketoquinone has great oxidizing powers in the presence of suitable hydrogen donors such as amino acids.

The part played by such an oxidizing system in the normal respiration of plant tissue has been a matter of dispute. Szent-Györgyi and Vietorisz (1931) concluded from their comparison between the rates of respiration of potato tissue containing whole cells and mashed tissue that the phenol oxidase system probably operated only in wounded tissue. The resulting *o*-quinone was valuable either in respect of its strong bactericidal properties or because of the mechanical protection it afforded the tissue through the formation of a precipitation membrane on the surface of the wound by interaction with the albuminoid substances present in the cells.

The results of an investigation of the part played by the catechol oxidase system in the respiration of slices of potato tuber are set forth in this paper. It is shown that this system is responsible for the major part of the O_2 uptake and of the CO_2 output. The tissue slice and manometric methods of Warburg and others have been employed. These methods have been of the greatest value in studying the intermediate stages in the respiration of animal tissues but have been little used with plant tissues. Caldwell and Meiklejohn (1937) record some results obtained with this method but do not give details of their technique. Details of the conditions under which reproducible results were obtained are given in the following section. While the observations recorded in detail were for slices of potato tuber other tissues which were examined in the same way were the fruit of the apple, the roots of carrot and turnip, the leaf of *Agave* sp., the spadix of *Arum maculatum*, and the petiole of rhubarb. Certain observations made on these tissues are recorded.

METHODS

After testing several varieties of potato it was found that the variety 'King Edward' was most suitable for the work. The tissue was cut into slices 0.03 in. thick and washed continuously in running tap-water which was mechanically stirred and aerated by a constant stream of air, thus preventing the develop-

ment of local anaerobic conditions in the washer. If anaerobic conditions develop there is a greater opportunity for the growth of bacteria and without bacterial infection potato tissue loses its activity irreversibly under anaerobic conditions. It was obvious from our first experiments that the tissue had to be washed for some considerable length of time if reproducible results were to be obtained and the rate of respiration to remain constant over any considerable period. The initial washing, done under the conditions described above, results in the starch and other contents of the cut cells on the surface of the tissue being washed away, the residual activity of the tissue being due to whole cells only. It also permits the wound reaction to become stabilized. Table I gives values for the rate of respiration of tissue washed for varying

TABLE I
Potato Slices, 0.03 in. thick. Phosphate Buffer pH 5.5
(m.ml. O₂ per gm. uptake dry wt. per hour)

Unwashed	.	407	426	—
Washed 2 days	.	616	579	560
" 3 "	.	520	559	577
" 4 "	.	514	562	—
" 5 "	.	590	550	529
" 6 "	.	575	572	544
" 7 "	.	586	589	—

lengths of time. The values in each column are for daily samples taken from one group of slices, each group having been cut at the same time from the same potato. It is clear that after the initial rise and fall in the rate of O₂ uptake, lasting for about two days, the rate of respiration of any mass of slices remains sufficiently constant over a further period of five days to permit of comparisons among experimental results obtained during this time. In addition to the marked effect which washing had upon the rate of O₂ uptake it also affected the R.Q. values. For unwashed tissues R.Q. values range between 0.7 and 0.8 and are very variable, while for washed tissue the values are very close to 1.0 and show little variation. The rise in the R.Q. values from 0.7 to 1.0 occurs after four hours' washing.

Carrot and turnip, like the potato, withstand long periods of washing without ceasing to respire normally; but tissues of the apple, rhubarb petiole, Arum spadix, and Agave leaf cease to do so if the period of washing extends over more than four days—probably due to the mechanical action of the stirrer.

The results obtained for any one tissue on any one day never varied by more than ± 2.5 per cent.; in the case of the carrot the values were always well within these limits and significance was attached to results which differed by greater amounts than these.

The experiments were all carried out at a temperature of $31^{\circ}\text{C.} \pm 0.05^{\circ}\text{C.}$; Novy (1925) considered this the optimum temperature for potato tissue.

In view of the low rate of respiration of plant as compared with animal tissue, the amount of the tissue used in the manometer cups in order to give

measurable changes of pressure during successive ten-minute intervals had to be considered. The figures in the following table show the rate of O_2 uptake (volume expressed as $l. \times 10^{-6}$) and the quantities of certain tissues which give useful changes in the manometric readings at short intervals. It is quite obvious, however, that while the following figures may serve as a rough guide nothing but experience of the technique can enable a suitable quantity to be taken each time without the labour of weighing each sample.

TABLE II

Tissue.	O_2 uptake (m.ml. per gm. dry wt. per hr.).	Amount of tissue (Dry weight).
Potato (tuber)	500–1000	100 mg.
<i>Arum maculatum</i> (spadix)	5000	20–30 "
Rhubarb (petiole)	2800	50 "
Agave (succulent leaf)	1500–2000	50–70 "
Carrot (root)	2100	60 "
Turnip (root)	2900	50 "

The necessity of using such large quantities of tissue results in an additional complication, namely the volume of tissue in the cup. If no account is taken of the tissue volume then the factors K_{O_2} and K_{CO_2} (Dixon, 1934) are incorrectly determined. It was found that the ratio tissue volume/dry weight of the slices of any one particular tissue was remarkably constant and that using this factor the tissue volume could be calculated with sufficient accuracy from the dry weight when the errors of the whole experiment were considered. The following calculation will indicate the importance of measuring the tissue volume if comparable results are to be obtained. Using potato slices of dry weight 300.4 mg. the volume of the tissue was 2.54 ml. giving, together with 5 ml. of buffer and 0.2 ml. 2N NaOH, a non-gaseous space of 7.74 ml. and a K_{O_2} value of 1.239. If the tissue volume be disregarded then the non-gaseous space is 5.20 ml. and K_{O_2} 1.484.

The rates of O_2 uptake by slices of potato tuber of different thicknesses were determined and the following table gives the values obtained.

TABLE III

Slice thickness	0.015 in.	0.03 in.	0.04 in.	0.05 in.
O_2 uptake (m.ml.)	612	618	498	476
		611		498

Similar tables were obtained for other tissues.

Steward (1932) has analysed the relationship between slice thickness and the respiration of tissue slices in great detail and has shown that the cells in a slice may be divided into two groups, those on the surface whose respiration is high due to the greater availability of oxygen and those in the interior whose respiration is much lower. He further showed that the outer active zone is very shallow. Our figures in Table III confirm these observations and suggest that the active zone is even shallower, in terms of the number of cells composing it, than he calculated, as even in slices six cells thick the inner

inactive zone is present. In view of the complication which is introduced by this inner zone all our measurements have been made with slices not thicker than 0.03 in.; such slices are four whole cells thick. In this work the problem of slice thickness is further complicated by the fact that many of the substances we have used do not penetrate in any great quantity beneath the surface layer of cells and that if the effect of inhibitors, &c. is to be correctly determined the diffusion of the substances used within the tissue and therefore the thickness of the slices used must be carefully examined. Caldwell and Meiklejohn (1937) used the tissue slice method with Barcroft differential manometers. The large standard deviation which they found with their material was probably due to their technique, in that the slices were not subjected to a preliminary period of washing to remove the contents of the cut cells and to allow the wound reaction to become stabilized, and the volume of the tissue within the cups was neglected. In particular the thickness of the sections is a matter of considerable importance as the figures in Table III show, that with very thin slices a very small increase in thickness may result in a great reduction in the O_2 uptake per gm. of dry weight.

The constitution of the buffer solution to be employed was studied. The following table shows the effect of concentration of buffer solution on the rate of O_2 uptake.

TABLE IV
Phosphate Buffer pH 5.5. Slices 0.03 in. thick

Strength of solution.	O_2 uptake. (m.ml./gm. dry wt./hr.)
Distilled water . . .	557
Buffer 0.067 M . . .	531
„ 0.044 M . . .	566
„ 0.013 M . . .	586
„ 0.003 M . . .	590

These values are for separate samples of tissue taken from the same mass.

A table showing the influence of pH value of the buffer solution on the uptake of O_2 is given below.

TABLE V
Phosphate Buffer, Concentration 0.013 M

pH.	O_2 uptake. (m.ml./gm. dry wt./hr.)
5.29	612
5.59	629
6.64	638
6.81	626
7.73	708
8.04	679

Separate samples from the same mass.

Between 5.59 and 6.81 there is no significant change in the O_2 uptake, the curve falls away slightly below 5.59 and the rise in the uptake at pH values

above 6.81 is in accord with the observations of Warburg (1930) on the effect of the —OH ion on the uptake of oxygen by animal tissues. In view, however, of the absence of any pronounced depressor effect upon the O_2 uptake of the tissue at pH 5.5 and of the great advantage of working at this pH when determining CO_2 output and R.Q. values, all the tissues used were tried at this pH value and it was found that in all cases no depressor effect was noticeable even after several hours.

The chemical constitution of the buffer solution used was found to be a factor controlling the rate of O_2 uptake. The following table illustrates the depressor effect of acetates even in dilute solutions.

TABLE VI
Slices 0.03 in. thick. Buffer pH 5.5

Concentration.	O_2 uptake (m.ml./gm. dry wt./hr.)	
	Phosphate.	Acetate.
0.067 M	583	—
0.04 M	—	240
0.013 M	605	—
0.008 M	—	462

This depressor effect is apparent with all the tissues tried and affects the O_2 uptake and CO_2 output equally, the R.Q. remaining at unity. The depressor effect is also apparent when the phosphate and acetate buffers are mixed.

In view of the long period of washing the danger of bacterial infection is considerable and such infection unless detected would seriously vitiate the results. It has been found that the detection of even a slight infection is easy, the rate of respiration rises rapidly with time, and in the course of three hours the rate may rise by as much as 50 per cent. over the initial value. If the infection is so small that it is difficult to detect it with certainty we have found that the addition of 0.5 ml. of 5 per cent. glucose or sucrose solution makes the matter clear. In the absence of any infection such additions have no effect on the rate of respiration, but with even the slightest infection the rate of respiration rises rapidly. The following table gives figures for infected tissue-potato.

TABLE VII

	11.40 p.m.— 12.40 a.m. m.ml. O ₂	2.20–3.20 p.m. m.ml. O ₂
Phosphate buffer only . . .	734	1021
Buffer and sucrose . . .	827	1222
„ and glucose . . .	863	1201

Similar results have been obtained with carrot. Turner (1937), Caldwell and Meiklejohn (1937) both report that uninfected slices of tissue, carrot in the first case and tomato stem in the second, showed a higher rate of respiration in the presence of sugar solutions than in inorganic buffer alone. We consider

that the difference between our observations and those of these workers is probably due to a difference in technique and, particularly in the case of Caldwell and Meiklejohn, a difference in the tissues used.

RESULTS

Slices of potato tuber 0.03 in. thick were used in 5 ml. of a phosphate buffer of pH 5.5. The CO_2 output and the O_2 intake were measured and after a short interval 0.5 ml. of a M/25 solution of catechol was added and the course of the respiration followed at 10-minute intervals. Fig. 1 shows the

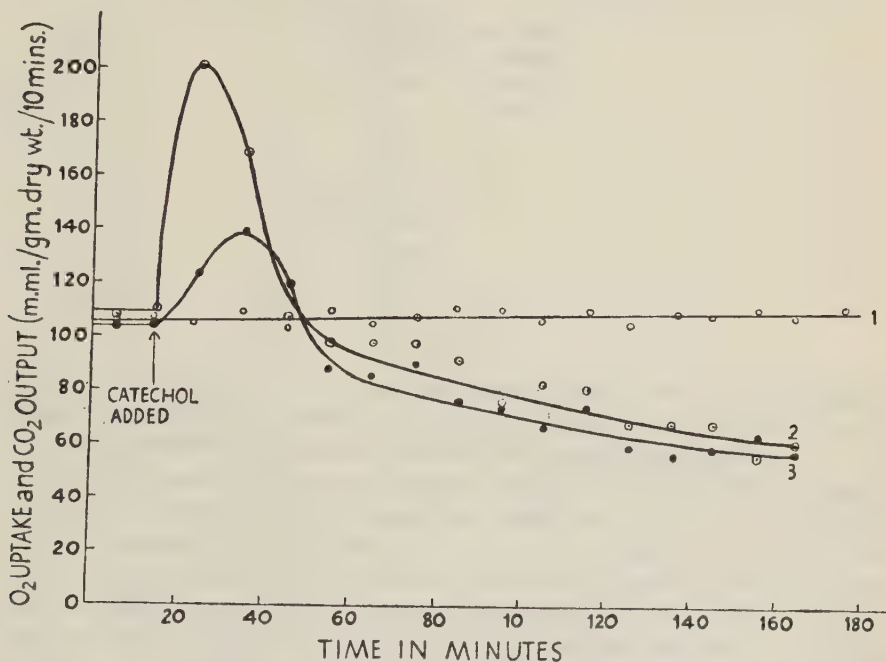


FIG. 1. Horizontal axis—time in minutes. Vertical axis— O_2 and CO_2 in m.ml./gm. dry wt./10 min. Graph 1. Phosphate alone— O_2 uptake. Graph 2. Phosphate and 0.5 ml. M/25 catechol— O_2 uptake. Graph 3. Phosphate and 0.5 ml. M/25 catechol— CO_2 output.

form of the curves obtained. Both the O_2 and CO_2 curves showed an initial rise above the 'phosphate only' line and this acceleration was followed by a depression which carried the values below this line, the R.Q. approaching unity.

By calculation on the basis that each molecule of catechol requires half a molecule of oxygen for oxidation to ortho-quinone it can be shown that out of a total 2.2 mg. of available catechol only 0.35 mg. were used. It appears, therefore, that the fall in the oxygen uptake and the CO_2 output is not due to the exhaustion of the oxidizable substrate. That it is probably due to the inhibition of the enzyme system is shown by adding a second portion of the catechol solution to the tissue when the depression due to the first is almost complete. Table VIII shows the result of this addition.

This inhibition of the enzyme action by the oxidation product of catechol confirms the work of Richter (1934) who, working with the enzyme *in vitro*, attributed the inhibition to the action of the ortho-quinone produced.

The factor influencing the percentage depression produced by the oxidation product of the catechol was then investigated. As the diffusion of the

TABLE VIII

<i>O</i> ₂ uptake	
Phosphate only.	(m.ml./gm. dry wt./ 10 min.) Catechol.
100	246
105	199
	89
	79
	68
	78
	Additional catechol.
	68
	68
	63
	68

catechol into the tissue was regarded as a possible limiting factor 0.5 ml. of M/25 catechol solution was added to slices of potato of different thicknesses and the depression measured. Fig. 2 shows the form of the curves obtained for the *O*₂ uptake of the slices of three different thicknesses, the thinnest being 0.015 in. The curves follow the general form of those in Fig. 1 but show certain interesting features when compared with one another. The first feature is that the thinner the section the greater the maximum value of the *O*₂ uptake following the addition of catechol, which suggests that only the surface cells are affected as the thinner the slice the greater is the ratio surface/volume for unit weight. It is therefore concluded that the oxygen uptake at any point on the curve is the sum of that by two groups of cells, namely those on the surface which have absorbed the catechol and those inside the tissue which have not. If the whole of the oxygen uptake of the cell is due to the activity of the oxidase system then the more nearly the slice approaches a two-cell thickness the more nearly will the inhibition of the *O*₂ uptake be complete. Table IX and Fig. 3 show the residual *O*₂ uptake as a percentage of the 'phosphate only' value with slices of different thicknesses. By measurement of a large number of cells the average length of a cell in the direction of the long axis of the tuber was calculated as being 0.0072 in. The sections used in the experiments were cut at right angles to the long axis of the tuber. In this case a slice 0.015 in. thick could not contain more than two whole cells in its depth. In sections of this thickness the residual *O*₂ uptake is still 33 per cent. of the initial 'phosphate only' value. As the sections

are only two cells thick, penetration by catechol might be regarded as being complete, all the cells being bathed on one surface by the catechol solution. From Richter's observation of the complete inhibition of the oxidase system

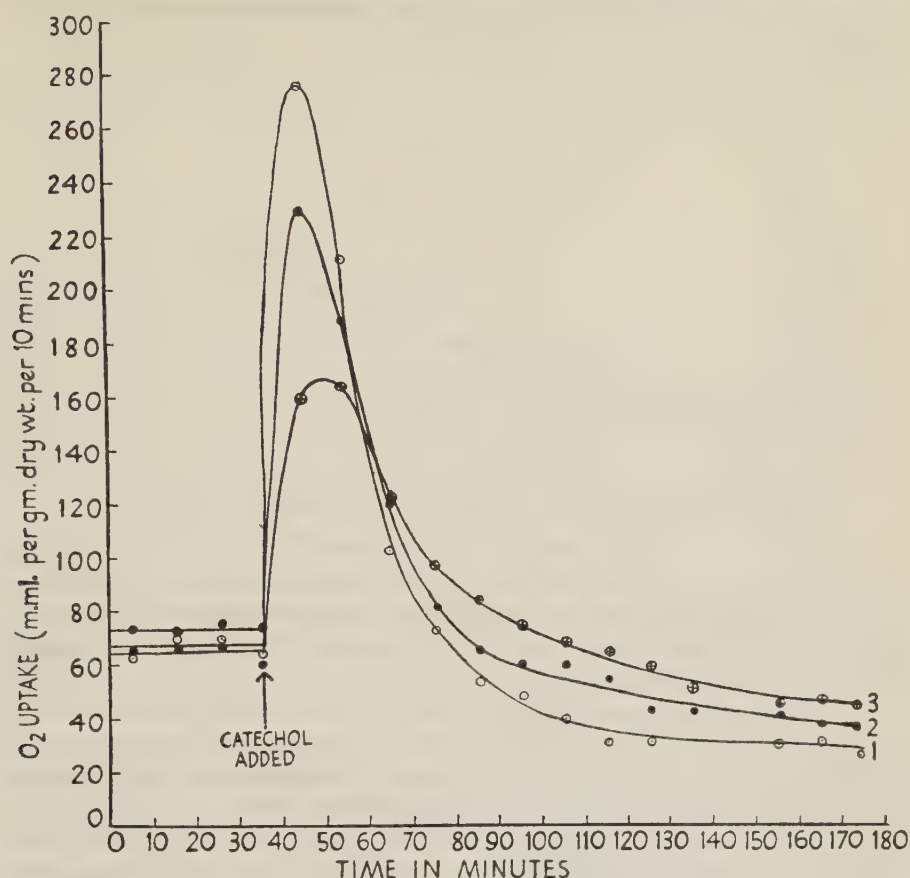


FIG. 2. Horizontal axis—time in minutes. Vertical axis—O₂ uptake, m.m.l./gm. dry wt./10 min. Action of 0.4 ml. M/25 catechol solution on O₂ uptake of potato slices of various thicknesses in phosphate buffer. Graph 1. Slices 0.015 in. thick. Graph 2. Slices 0.025 in. thick. Graph 3. Slices 0.035 in. thick.

by the oxidation product of catechol it may be concluded that in such thin slices the part of the O₂ uptake which is the product of the oxidase system

TABLE IX

Slice thickness (in.)	0.05	0.04	0.03	0.025	0.02	0.015
Residual O ₂ uptake as per cent of 'phosphate only'	74	58	48	42	38	33

would be inhibited and that any residual uptake must be due to a system in which catechol oxidase plays no part. In these slices of potato tissue it would appear that 33 per cent. of the O₂ uptake is due to a system not involving

the catechol oxidase system. It is important to note that the tissue slices retain their turgidity under all concentrations of catechol used and throughout the duration of the longest experiments. Parallel experiments on the CO_2 output show that it is similarly inhibited in slices of 0.015 in. thickness and

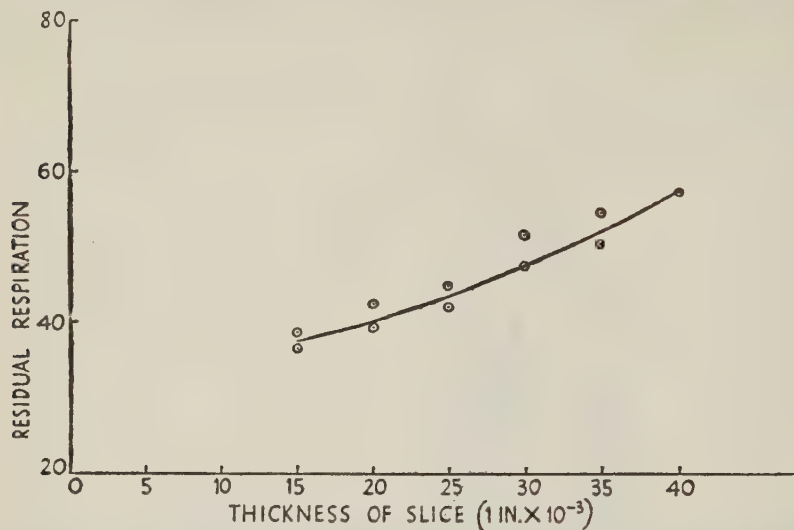


FIG. 3. Horizontal axis—slice thickness (in. $\times 10^{-3}$). Vertical axis—residual respiration after addition of catechol expressed as a percentage of original 'phosphate only' respiration.

that the R.Q. of the residual respiration is 1.0 even at the end of the longest experiments of five hours duration.

Richter (1934) has shown that the inhibiting effect of the oxidized catechol can be removed by the addition of aniline to precipitate the inhibitor as an anilino-orthoquinone. It was found by experiment that aniline had no effect on the rate of respiration, but when added to the tissue in the presence of catechol did not inhibit the depressor effect, very irregular results were obtained, and the flaccid tissue after only very short experiments suggested that the mixture had had a toxic action on the tissue activity.

An extract containing an *o*-dihydroxy compound has been isolated from potato tubers by several workers, including Onslow, and from experimental work *in vitro* has been regarded as part of a phenol oxidase system. Following Onslow's (1919) directions we isolated this compound using 95 per cent. alcohol, the solution was reduced to small bulk *in vacuo*, the required fraction was precipitated from aqueous solution using lead acetate and the lead compound decomposed with the calculated amount of 10 per cent. H_2SO_4 . The filtrate after the removal of the precipitate of lead sulphate was evaporated down *in vacuo* and yielded a pale yellow gum which showed no signs of crystallizing after being kept over calcium chloride *in vacuo* for several months, but under such conditions it lost a considerable part of its activity both when used in the experiments described below and when tested with

solutions of ferric chloride and sodium carbonate with which it has been described as giving certain colour reactions. The aqueous solution of the phenolic compound was prepared by dissolving an arbitrary amount of the gum in distilled water, making up to a known volume and adjusting the pH to approximately 5.5 by the addition of dilute soda solution. 0.1 ml., 0.2 ml., 0.5 ml. were added to slices of potato tuber respiring in phosphate buffer pH 5.5, and Fig. 4 shows the form of the curves obtained for the O_2 uptake.

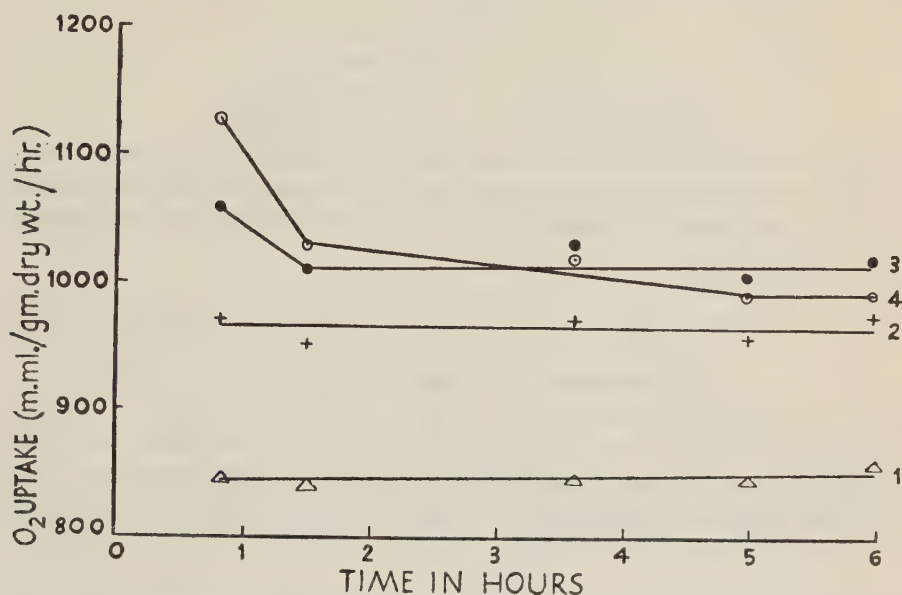


FIG. 4. Horizontal axis—time in hours. Vertical axis— O_2 uptake. m.ml./gm. dry wt./hr. Graph 1. Phosphate alone. Graph 2. Phosphate and 0.1 ml. extract. Graph 3. Phosphate and 0.2 ml. extract. Graph 4. Phosphate and 0.5 ml. extract.

The curve for tissue and 0.1 ml. of extract was constant at a value about 116 per cent. of the 'Phosphate only' value and remained at that level throughout the experiment, the curve for the addition of 0.2 ml. fell slowly in the early stages but reached a constant value 121 per cent. of the 'phosphate only' value, the curve for the addition of 0.5 ml. fell rapidly and attained a constant value at 119 per cent. of the 'phosphate only' value. It is of interest to note that whatever the initial value of the O_2 uptake the constant level attained was practically the same for all the amounts added, the differences between the final values being hardly significant.

Onslow (1919) purified the extracted material by submitting it to ether extraction. We have treated our gummy extract in a similar manner and obtained as a result two fractions, one ether soluble and the other insoluble. The ether soluble when dissolved in water gave a green colour with ferric chloride but no colour reaction with a solution of sodium carbonate, while the insoluble fraction gave positive colour reactions with both the above

reagents. The insoluble fraction is much less stable when exposed to air than the soluble: it becomes dark brown in colour after standing for several months in the air, while the ether soluble retains its pale yellow colour. Both extracts were tested by adding them to potato slices respiring in a phosphate buffer. The ether soluble extract had no effect upon either the O_2 uptake or CO_2 output, as is shown in Table X.

TABLE X

	O_2 m.ml.	CO_2 m.ml.	R.Q.
Before addition	934	962	1.03
After „	943	963	1.02

The ether insoluble extract had, on the other hand, a pronounced accelerating effect upon both the rate of O_2 uptake and CO_2 output, both curves reaching a constant value much in excess of the 'phosphate only' values after a short interval. (Table XI.)

TABLE XI

	O_2 m.ml.	CO_2 m.ml.	R.Q.
Before addition	1542	1588	1.03
After „	1750	1923	1.10

Table XII gives the values of O_2 uptake and CO_2 output when the whole extract before ether extraction was added to respiring potato slices. The values recorded were maintained throughout the duration of the experiment lasting several hours.

TABLE XII

	O_2 m.ml.		CO_2 m.ml.		R.Q.	
Buffer alone . .	1402	1385	1444	1426	1.03	—
„ and extract . .	2088	2000	2302	2193	1.10	1.09

Fig. 5 illustrates the form of the curve obtained by following the O_2 uptake and CO_2 output after adding the extract (0.4 ml.) to slices respiring in phosphate buffer and taking readings at 10-minute intervals. On addition of the extract the O_2 uptake values rose gradually to a maximum value and then fell away slowly and attained a constant value well above the 'phosphate only'. The CO_2 output as shown by the changes in manometric pressure followed a different course and only attained a maximum value above that of 'phosphate only' after a long period, the R.Q. finally reaching a value of 0.94. This slow rise in the rate of CO_2 output is really illusory, being due, not to a low rate of output of CO_2 , but to the binding of the CO_2 by some substance in the added extract. When this CO_2 was liberated by the addition of dilute acid at intervals during the respiratory measurements then the initial CO_2 output was found to be large, to follow rapidly upon the addition of the extract and to attain a maximum value in excess of the O_2 uptake. It is of interest to note

that in the CO_2 output curve the maximum is attained at an earlier point than that in the O_2 intake curve. These experiments appear to show that there is present in the cells of the potato tuber an enzyme which is not only capable of oxidizing catechol but also an *o*-dihydroxy phenol present in the tissue, and that such oxidations are accompanied by an output of CO_2 .

It was desirable to establish whether or not the two substances were oxidized by one and the same enzymic system. To test this, 0.4 ml. of the

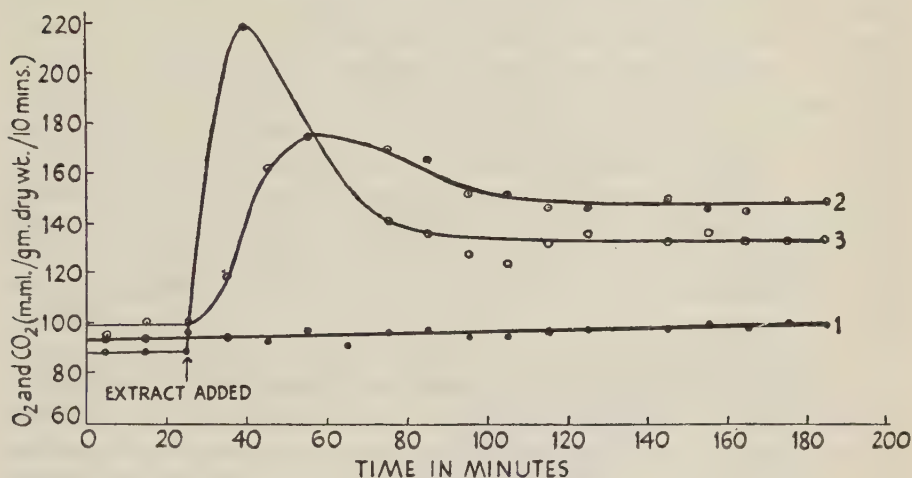


FIG. 5. Horizontal axis—time in minutes. Vertical axis— O_2 uptake and CO_2 output. m.m.l./gm. dry wt./10 min. Graph 1. Phosphate only, O_2 uptake. Graph 2. Phosphate and extract. O_2 uptake. Graph 3. Phosphate and extract. CO_2 output. Bound CO_2 included only in the 30–50 period.

solution of the phenolic compound were added to the potato slices respiring in a phosphate buffer and the O_2 uptake was followed until a constant value was obtained. Then 0.4 ml. of M/25 solution of catechol was added, the rate of O_2 uptake increased to a high value and then fell, ultimately passing below the 'phosphate only' line, the percentage depression being of the same value as that obtained when catechol was added without previous addition of the extract. Fig. 6 illustrates the form of the curve obtained. When the extract was added after the addition of catechol had reduced the O_2 uptake to a low value, no increase in the O_2 uptake resulted. It was therefore concluded that only one enzymic system was involved in the oxidation of the two substrates.

DISCUSSION

It is clear that the cells of the potato tuber contain an enzyme capable of oxidizing catechol *in vivo*. From the red colour of the compound formed between aniline and the oxidation product it would appear that this oxidation product is an *o*-quinone. The depressor effect that this product of catechol oxidation exerts upon the normal respiration of the tissue shows that the normal respiration involves the activity of the catechol oxidase enzyme. By

using slices of two whole cell thickness it has been possible to determine that 66 per cent. of the total respiratory activity is dependent upon the oxidase system. It is of interest that both parts of the respiration process, that involving and that not involving the oxidase system, have an R.Q. of 1.0. The fact

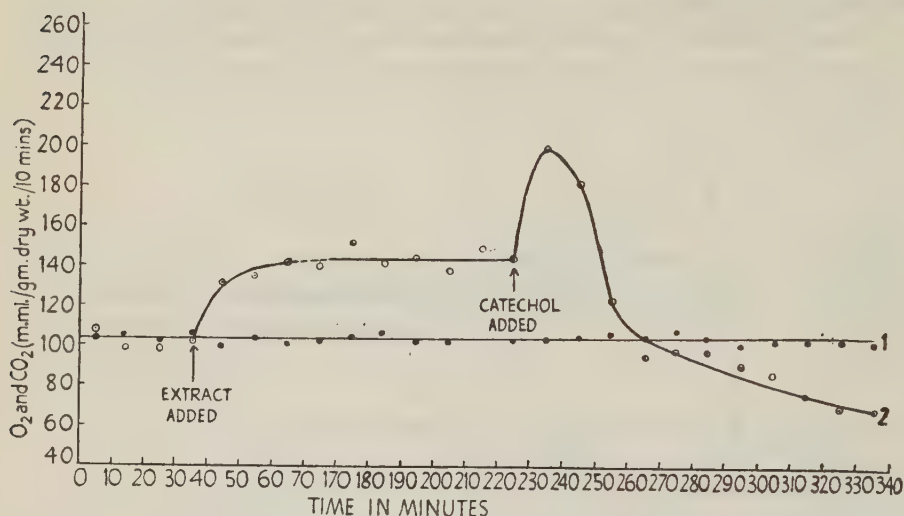


FIG. 6. Horizontal axis—time in minutes. Vertical axis—O₂ uptake and CO₂ output. m.m.l./7 gm. dry wt./10 min.

that the addition of catechol to the respiring tissue not only raises the O₂ uptake but also the CO₂ output shows that the oxidized catechol is capable of taking part in some system which results in the production of CO₂. It also indicates that under normal conditions there is present in the slices an excess of the substance with which the oxidized catechol reacts, in other words that the rate CO₂ output is limited by the amount of the normally occurring oxidizing agent.

As the catechol oxidase system appears to play a part in the respiration of the slices of tuber and as a phenolic compound has been isolated from the tuber, the part played by this compound was determined by adding it to the respiring tissue. The result of adding the extract was an increase in the rate of CO₂ output and of O₂ intake. Where small amounts of the extract were used then the O₂ intake curve rose gradually to a maximum value and continued at that level; where large amounts were added then the maximum value reached was not maintained but the curves fell away to a constant value of about the same magnitude as that reached when smaller quantities were added. This suggests that when large amounts of the extract are added some other factor becomes the limiting factor in the rate of respiration.

The difference in the form of the O₂ uptake curves after the addition of a large quantity and after the addition of a small quantity of the extract can be explained as follows. As the amount of the phenolic body added to the

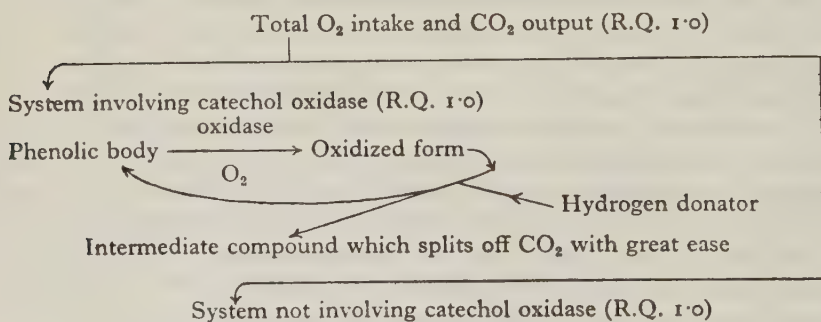
respiring slices is limited the factor controlling the rate of oxygen uptake over a long period of time, will be the rate at which the oxidized body is reduced to a form ready for re-oxidation, in other words the amount of oxidizable substrate will be the controlling factor. From the form of the CO_2 output curve after the addition of catechol, it was deduced that under normal conditions there was an excess of this oxidizable substrate present in the cells. On the addition of the extract the uptake of O_2 is increased as the extract is oxidized; where a large quantity of the extract was added the O_2 uptake rises to a high value, but this value cannot be maintained, as owing to the limited amount of oxidizable substrate present the oxidized phenolic body is being produced at a greater rate than it is being reduced and an excess of the oxidized phenolic compound is accumulating. The rate of O_2 uptake therefore falls away and becomes stabilized at a value which corresponds to the rate at which the oxidized phenolic compound is being reduced, i.e. the rate at which the oxidizable substrate is produced. When small quantities of the extract are added they are insufficient to oxidize all the oxidizable substrate and therefore the rate of O_2 uptake rises to a maximum value which it maintains, representing the rate at which the amount of phenolic body added can oxidize the oxidizable substrate produced by the cells. When excess of the phenolic body is added then the output of oxidizable substrate by the cells limits the rate of respiration, but with smaller quantities the rate is limited by the amount of the phenolic compound present. The CO_2 curves can be interpreted similarly. As the O_2 intake and the CO_2 output remain constant during the whole experiment once the initial acceleration is over, and this constant value is much in excess of 'phosphate only' values, it would appear that the phenolic body has entered into some cyclic system, being alternately oxidized and reduced; in the former stage being responsible for the O_2 uptake of the tissue and in the latter reacting with some substance produced by the cells resulting in the reformation of the phenolic compound and the formation of some substance which readily splits off CO_2 . This oxidizable substrate formed by the cells acts as a hydrogen donor and together with the oxidized phenolic compound forms a dehydrase system. The rapid output of CO_2 when the phenolic compound is added in large quantity to the respiring tissue, and the fact that this CO_2 is produced before the oxidation of the compound is proceeding at a very rapid rate, is probably due to a considerable part of the phenolic compound being in an oxidized state before being added. This oxidized portion reacts with the hydrogen donor with the liberation of CO_2 . When the amount of phenolic compound added is large then the whole of the accumulated hydrogen donor is removed rapidly and the rate of CO_2 output falls to the level corresponding to the rate at which the plant cells are able to produce it. When the amount added is small then the rate of CO_2 production gradually attains to a level controlled by the amount of phenolic compound present. It is probable that a considerable portion of the phenolic compound in aqueous solution is in an oxidized state when the

instability of these dihydroxy compounds is considered. Catechol itself when in aqueous solution, particularly if shaken in contact with the air, becomes dark brown through oxidation. The aqueous solutions of the phenolic compounds are always dark brown in colour even when kept for only a short time, and after the solution has been shaken in the side arm of a manometer cup at 30° C. for some time it is reasonable to suppose that considerable oxidation has taken place. The following observation strengthens this explanation. In experiments where an aqueous solution of the extract, which has been allowed to stand for many days in the open air before use and has attained a deep brown colour, has been added to respiring tissue the CO_2 output during the first twenty minutes reached a very high value while the O_2 intake remained at almost the 'phosphate only' value. At the end of this period the O_2 intake value started to rise and gradually attained a maximum value, at which it remained constant during the remainder of the experiment. The CO_2 curve fell from its initial high value and attained a constant level such that the R.Q. was unity.

The nature of the agent which binds the CO_2 in the early stages after the addition of the extract is unknown, but it is unlikely that it is in the form of bicarbonate as the pH of the solution precludes the formation of this substance in any quantity.

It may be noted that the R.Q. values for the extract and tissue respiration experiments as shown in Table V are in excess of unity, being about 1.1. This can be ascribed to the CO_2 liberated by the reaction between the hydrogen donator in the tissue and the oxidized phenolic compound in the added extract, the oxidized substances not being formed in the course of the experiment but being already present when the extract was added, so that the CO_2 which is liberated has no counterpart in the O_2 uptake values.

The scheme for the respiration of the slices of potato tuber is as follows:



SUMMARY

The conditions under which the tissue slice and manometric technique of Warburg and others can be successfully applied to plant tissues have been determined. Details of the work on slices of potato tuber are recorded. Other

tissues which have been used are the fruit of the apple, the roots of carrot and turnip, the leaf of *Agave* sp., the spadix of *Arum maculatum*, and the petiole of rhubarb. Following the methods described, values for the rate of respiration of any one tissue on any one day lie within ± 2.5 per cent.

Using this technique the position of the catechol oxidase system in the respiration of slices of potato tuber has been studied. It has been concluded that a system involving an oxidase, a phenolic compound, and a dehydrase is concerned in two-thirds of the total respiratory gaseous exchanges of the slices of potato tuber, those of the remaining one-third are dependent on some other system. For both parts the respiratory quotient is unity. The oxidized phenolic compound reacts with a hydrogen donor with the formation of a substance which splits off CO_2 with great ease.

The thanks of one of us (G. C. W.) are given to the Council of this University for the grant of a scholarship during the tenure of which this work was carried out. We express our thanks to Dr. H. A. Krebs who extended to us the hospitality of his laboratory during our earliest investigations and to Professor B. H. Bentley who made this work possible and who has facilitated it in every way.

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Physiological Studies in Plant Nutrition

IX. The Effect of Mineral Deficiency on the Fructosan Metabolism of the Barley Plant

BY

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With four Figures in the Text

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INTRODUCTION

THE isolation by Archbold and Barter (1935) of a fructosan from the leaves of barley aroused special interest in these substances. Accordingly Archbold (1938) developed a technique for the estimation of fructosans and determined the fructosan content of leaves, stems, and ears throughout the life-history of the plant (1938a). Hitherto fructosans had been shown to occur (Belval, 1924; Colin, 1925) in stems and ears but not in leaves. Archbold's work shows that these substances are more widely distributed in the plant and form a larger fraction of the soluble carbohydrate than was previously realized.

To ascertain further the role of fructosan it was desirable to determine what conditions favour their formation, and attention was therefore directed to the effect of mineral deficiency on fructosan content. Archbold (1938a) has already shown that fructosans are particularly abundant under conditions of low nitrogen. In the present investigation the effect of a wider range of nutrient deficiency has been studied. Varying levels of potassium and phosphorus and of the balance of sodium and calcium have been employed, nitrogen being always maintained at a high level. The concentration of the different water-soluble carbohydrates has been determined throughout the plant.

This communication deals exclusively with fructosan metabolism, and only such data as are relevant to this question will be considered.

FIELD EXPERIMENTAL PROCEDURE

The method of sand culture which has been described in several previous publications from this Institute (Gregory and Crowther, 1928; Richards and Templeman, 1936) was again employed. The plant used was a pure line of barley, variety Plumage Archer, the seed having been sown on May 4, 1936. For the complete experiment some 700 separate cultures were required. The work was carried out in the open at Rothamsted Experimental Station.

The manurial scheme is given in Table I. Three levels of potassium, two

TABLE I
Manurial Scheme

HAK ₁	HCK ₁	LAK ₁	LCK ₁	
HAK ₃	HCK ₃	LAK ₃	LCK ₃	
HAK ₅	HCK ₅	LAK ₅	LCK ₅	
where H represents phosphorus at high level				
L	”	”	reduced to 1/5	
A	”	high sodium with low calcium		
C	”	high calcium with no sodium		
K ₁	”	potash at high level		
K ₃	”	”	reduced to 1/9	
K ₅	”	”	1/81	
Nutrients added (gm. per pot):				
	HA	LA	HC	LC
NaNO ₃	9.749	9.749	—	—
Na ₂ HPO ₄	1.274	0.258	—	—
Na ₂ SO ₄ . 10H ₂ O	—	2.158	—	—
Ca(NO ₃) ₂ . 4H ₂ O	—	—	13.535	13.535
CaH ₄ (PO ₄) ₂ . H ₂ O	—	—	1.057	0.211
CaCl ₂ . 6H ₂ O	0.37	0.37	0.37	0.37
MgSO ₄ . 7H ₂ O	1.25	1.25	1.25	1.25
MnSO ₄ . 4H ₂ O	0.10	0.10	0.10	0.10
FeCl ₃	0.15	0.15	0.15	0.15
	K ₁	K ₃	K ₅	
K ₂ SO ₄	1.850	0.206	0.023	

levels of phosphorus, and two different sodium-calcium ratios were used in all possible combinations, making twelve treatments. This arrangement allows a statistical analysis of the various effects and interactions to be made. The appropriate nutrient was applied in solution ten days after sowing when germination was complete. The plants were watered with tap-water in dry weather, while in wet weather leaching and waterlogging was prevented by the use of portable transparent screens. A description of the morphological characteristics of the plants will be published later; suffice it to say here that large differences in growth and well-marked deficiency symptoms appeared. Gregory (1937) has summarized much of the available information concerning these effects.

SUGAR DETERMINATION

Sampling. Entire plants were sampled on three occasions, the first sample being made on June 5–8, when the third leaf on the main axis was mature and before stem elongation had occurred. The second was taken on June 19–24, when the sixth leaf on the main axis was mature, stem elongation was at an early stage, and vegetative growth was maximal. The third was taken on July 9; stem elongation was then rapid but ears had not yet emerged. Owing to lack of material the first sample, for which six plants per treatment were required, was not replicated, while in the second and third samples two replicates, consisting of three plants each taken at random, were made. Leaves, stems, and roots were treated separately.

In addition to these 'whole plant' samples each leaf on the main axis was sampled as it reached maturity. A detailed picture of manurial effect throughout the growing period is thus obtained. As these single-leaf samples show the same general effects of manurial deficiency on fructosan content as do the large samples, it is unnecessary to discuss them here.

Leaves. Leaves were weighed immediately on removal from the plant and were then cut into short lengths and killed in boiling alcohol. Subsequently they were extracted with cold water and analysed as described by Archbold (1938a). Reducing sugars were determined in charcoal-cleared solutions, and 0.2 N sulphuric acid was used to hydrolyse sucrose and fructosan. Fructosan was calculated as 100/88ths of the excess of fructose over glucose produced by hydrolysis (Archbold 1938, p. 199), the small percentage of glucose combined in fructosan being thus included in the estimate.

Stems. It was found that the cold-water method was not adequate for the extraction of stems, especially in the third sample with much lignification. When, however, the material was first coarsely ground at least 97 per cent. of the total sugar could be extracted. The results of tests with fully lignified material are given in Table II. As no mill suitable for grinding such tissue quantitatively was available the apparatus described in the appendix to this paper was devised. It appears that extraction is facilitated if the material has previously been kept in alcohol for a lengthy period. The tests recorded in Table II were carried out on material which had remained in alcohol for only a short period, consequently the errors shown are maximal. Apart from the milling process stem material was extracted and analysed by the same methods as were leaves.

Roots. Roots were washed free of sand, drained of superficial water, then preserved and analysed by the same method as used for stems and leaves. In many cases, especially in the first two samples, the increase in glucose after hydrolysis with 0.2 N acid exceeded the increase in fructose. In some cases excess of glucose was greater than the total fructose set free by hydrolysis. Consequently it was impossible, by the methods employed, to obtain any estimate of fructosan, and no analytical data for roots will be presented in this

paper. The presence of a fructosan in certain treatments was, however, established, but it will be necessary to distinguish between sucrose and other substances which yield glucose on hydrolysis before this question can be

TABLE II
The Effect of Grinding on the Extraction of Sugars from Barley Stalks

Sugar Extracted					Percentage left after 1st extraction.	
I.					II.	Total.
Material crushed before 1st extraction:						
A	.	.	10.75	0.15	10.90	1.4
B	.	.	9.36	0.28	9.64	2.9
Material not crushed before 1st extraction:						
C	.	.	7.94	2.19	10.13	21.7
D	.	.	7.35	2.84	10.19	27.8

I. 1st extraction: material shaken with water for three hours. II. 2nd extraction: material dried and finely milled before shaking with water.

elucidated further. It has been shown that the substance in question is not starch.

In contrast with leaves and stems the roots showed a relatively high reducing sugar content, amounting, on the average of all treatments, to more than 50 per cent. of the total sugar present. Consequently, even if a large proportion of the fructose yielded by hydrolysis was derived from fructosan, the ratio of fructosan to total sugar in roots would be much lower than in stems. The present evidence suggests that fructosan is more abundant in the low phosphorus treatments and accumulates between the second and third samples. These indications in roots are similar to the effects found in stems and leaves which are described below.

EXPERIMENTAL DATA

The analytical results are given in Table III, fructosan and total soluble sugar being expressed as percentages fresh weight. The ratio of fructosan to other sugars is also given. The data for the replicated samples have been analysed statistically (see Table VIII) and only such effects as are statistically significant will be considered. The first sample being unreplicated was omitted from the statistical analyses and will be disregarded in this discussion except when the mean effect of sampling date is considered. As the effect of treatment is in general similar in leaves and stems it is convenient to examine the two sets of data together.

A. Concentration of fructosan.

The *mean effects* of the factors are shown in Table IV. It will be seen that fructosan level is markedly lowered by potassium deficiency, particularly in the

TABLE III
Concentrations (% Fresh Weight) of Fructosan and Total Sugar of Barley Plant

	FIRST SAMPLE						SECOND SAMPLE						THIRD SAMPLE					
	LEAVES			STEMS			LEAVES			STEMS			LEAVES			STEMS		
	Fruct.	T.S.	f.	Fruct.	T.S.	f.	Fruct.	T.S.	f.	Fruct.	T.S.	f.	Fruct.	T.S.	f.	Fruct.	T.S.	f.
HAK1	0.45	2.07	0.28	0.27	0.92	0.41	0.41	2.22	0.22	0.55	1.81	0.43	0.86	2.74	0.45	3.37	5.85	1.38
HAK3	0.18	1.25	0.16	0.24	0.93	0.35	0.82	2.45	0.49	1.11	2.45	0.83	1.06	2.05	0.67	3.46	5.41	1.86
HAK5	0.11	0.67	0.19	0.22	0.55	0.66	0.12	1.33	0.10	0.19	1.01	0.23	0.02	1.02	0.02	1.10	2.35	0.80
HCK1	0.00	2.48	—	0.00	0.61	—	0.14	1.19	0.14	0.20	1.20	0.20	0.00	0.74	—	0.39	1.25	0.45
HCK3	0.79	2.42	0.50	0.09	0.89	0.11	0.57	1.78	0.23	0.00	0.38	—	0.00	0.73	0.05	0.00	0.58	—
HCK5	0.51	2.96	0.70	0.10	1.11	0.09	1.30	1.71	0.18	0.14	0.98	0.16	0.57	2.81	0.25	2.41	5.06	0.80
LAK1	0.27	1.78	0.18	0.00	0.49	—	0.25	0.80	0.00	0.00	1.10	0.19	0.86	2.99	0.41	3.31	5.45	1.56
LAK3	0.00	0.74	—	0.19	0.88	0.28	0.25	1.74	—	0.00	2.38	0.12	0.10	2.14	0.05	1.85	3.85	0.88
LAK5	0.18	0.87	0.25	0.00	0.56	—	0.74	1.74	—	0.03	0.83	0.04	0.95	3.05	0.45	2.10	4.00	1.22
LOK1	0.19	1.92	0.11	0.20	0.87	0.50	0.00	1.73	0.18	0.00	1.03	0.00	0.00	1.53	0.10	0.00	0.92	—
LOK3	0.52	2.29	0.20	0.24	0.96	0.33	0.47	2.14	0.28	0.57	1.56	0.54	2.52	5.45	0.85	3.70	5.82	1.86
LOK5	0.51	2.19	0.20	0.33	1.31	0.33	0.10	2.33	0.04	0.80	1.88	0.72	0.73	4.58	0.80	3.43	5.32	1.78
							0.14	2.68	0.06	0.80	1.83	0.79	0.00	2.02	—	1.68	2.04	1.32
							0.00	0.19	—	0.00	0.63	—	0.30	1.91	0.10	0.75	2.80	0.49
							0.20	0.99	0.25	0.20	0.91	0.28	0.23	1.16	0.25	0.18	1.80	0.72
							1.31	2.85	0.16	1.41	2.68	1.08	1.31	3.68	0.56	2.62	4.68	1.27
							0.83	3.20	0.60	2.35	3.95	1.44	1.47	3.32	0.79	2.60	4.12	1.86
							1.15	4.08	0.25	1.82	3.76	0.92	1.85	5.22	0.54	2.33	4.70	1.00
							0.53	4.23	0.37	1.99	3.78	1.08	2.48	5.57	0.70	2.86	4.88	1.44
							0.73	3.21	0.22	0.91	2.28	0.69	0.16	1.91	0.09	0.35	1.83	0.23
							0.73	3.21	0.28	1.17	2.63	0.79	0.32	1.77	0.23	0.19	1.36	0.16

f = Ratio of fructosan to other sugars
 K1 = High potassium
 K3 = Medium potassium
 K5 = Low potassium

Fruct = Fructosan
 H = High phosphorus
 L = Low phosphorus
 T.S. = Total (soluble) sugar
 A = High sodium
 C = High calcium

stems. Phosphorus deficiency causes fructosan concentration to increase, the relative increase being greater in leaves. A smaller increase is shown by the high calcium over the high sodium treatments. Especially in stems, fructosan

TABLE IV
Mean Effect of Various Factors on Fructosan Concentration
(% Fresh Weight)

Potassium			Phosphorus		
	Leaves.	Stems.		Leaves.	Stems.
High .	0.93	2.02	High .	0.28	0.86
Medium	0.53	1.19	Low .	0.82	1.44
Low .	0.18	0.24			

Sodium-Calcium Balance			Sampling Date		
	Leaves.	Stems.		Leaves.	Stems.
High sodium .	0.45	1.01	1st sample .	0.31	0.15
High calcium .	0.65	1.29	2nd sample .	0.34	0.65
			3rd sample .	0.75	1.65

concentration increases up to the last sample. It will be noted that after the first sample fructosan concentration is higher in stems than leaves.

The various *significant interactions* for leaves and stems are shown in Table V. The interactions involving potassium are given in the upper part of the

TABLE V
Fructosan as Percentage Fresh Weight: Significant Interactions in
Leaves and Stems

I. Interaction of potassium with sodium-calcium balance.							
II.	"	potassium with sampling date.					
III.	"	potassium with sodium-calcium balance and sampling date.					
IV.	"	phosphorus with sodium-calcium balance.					
V.	"	phosphorus with sampling date.					
VI.	"	phosphorus with sodium-calcium balance and sampling date.					

		High potassium		Medium potassium		Low potassium	
		Leaves.	Stems.	Leaves.	Stems.	Leaves.	Stems.
I	{ High sodium .	1.07	2.17	0.16	0.73	0.12	0.14
	{ High calcium .	0.78	1.89	0.96	1.65	0.24	0.33
II	{ Second sample .	0.50	0.92	0.31	0.75	0.22	0.29
	{ Third " .	1.35	3.13	0.76	1.64	0.15	0.18
III	{ Second sample { high sodium .	0.48	0.82	0.12	0.50	0.11	0.05
	{ high calcium .	0.52	1.02	0.50	1.00	0.32	0.53
	{ high sodium .	1.65	3.51	0.19	0.97	0.13	0.23
	{ Third " { high calcium .	1.06	2.76	1.34	2.31	0.16	0.14

		High phosphorus		Low phosphorus	
		Leaves.	Stems.	Leaves.	Stems.
IV	{ High sodium .	0.31	0.86	0.59	1.16
	{ High calcium .	0.25	0.85	1.05	1.72
V	{ Second sample .	0.18	0.21	0.50	1.09
	{ Third sample .	0.38	1.51	1.14	1.80
VI	{ Second sample { high sodium .	0.28	0.34	0.19	0.57
	{ high calcium .	0.07	0.08	0.81	1.61
	{ high sodium .	0.32	1.39	0.99	1.76
	{ Third " { high calcium .	0.43	1.63	1.28	1.84

table, the other interactions being given below. The numbering of the various sections of the table corresponds to the numbering of the following paragraphs. Three interactions which are significant, though not highly so, in leaves alone are considered later.

(i) *Potassium and the sodium-calcium balance.* The lowering of fructosan level due to extreme potassium deficiency is much less in the high calcium than the high sodium treatments. Medium potassium deficiency causes a marked reduction in the high sodium treatments, but in the high calcium treatments a small increase is found in leaves and a small decrease in stems. Consequently the general level of the high calcium treatments is higher than that of the high sodium treatments, although at the high potassium level the high sodium values are greater.

(ii) *Potassium and sampling date.* With advancing age the lowering in fructosan level due to potassium deficiency becomes more marked.

(iii) *Potassium, sodium-calcium balance, and sampling date.* In treatments with high calcium and low potassium there is a marked decrease in fructosan between the second and third samples, while in the remaining treatments there is a considerable increase, especially at the high potassium level. The interaction of age and potassium deficiency (see (ii) above) is now seen to be most marked in the high sodium treatments. In leaves the medium potassium value at the high calcium level exceeds the high potassium value in the last sample only.

(iv) *Phosphorus and the sodium-calcium balance.* The effect of phosphorus deficiency leading to increase in fructosan content is greater in the high calcium than in the high sodium treatments. Hence, whereas at the high phosphorus level fructosan content is little affected by the sodium calcium balance, under low phosphorus the high calcium treatments show markedly higher values.

(v) *Phosphorus and sampling date.* In leaves the effect of phosphorus level is greater in the third than in the second sample; in stems, on the other hand, phosphorus occasions maximum differences in the second sample. Thus whereas in stems the accumulation of fructosan between the second and third samples is encouraged by high phosphorus, in leaves low phosphorus causes a greater accumulation.

(vi) *Interaction of phosphorus, sodium-calcium balance, and sampling date.* It has already been shown ((iv) above) that the effect of phosphorus is greater with high calcium than with high sodium, and ((v) above) that this effect changes markedly between the second and third samples. The present interaction shows these two effects to be interdependent. For leaves of the high sodium treatments fructosan level is, at the time of the second sample, greater at the high phosphorus level. This is the only case in which phosphorus deficiency fails to cause an increase in fructosan level. In the high calcium treatments, on the other hand, leaves of both samples show a similar increase due to phosphorus deficiency. A different relation holds in stems, in which the increase of fructosan due to phosphorus deficiency in the high calcium treatments is especially marked in the second sample and much diminished at the third sample. In

stems the high sodium treatments show a much smaller effect of phosphorus deficiency at the second sample and a slightly greater effect at the third sample.

In addition to the effects discussed above, the interactions of potassium with phosphorus, of potassium with phosphorus and the sodium-calcium balance, and the interaction of all factors together are all significant, though not highly so, for leaves alone. At the low phosphorus level the values for potassium deficient leaves are much higher relative to the high potassium values than at the high phosphorus level (HK₁ 0.62; HK₃ 0.17; LK₁ 1.23; LK₃ 0.91).¹ This differential effect of phosphorus on the symptoms of potassium deficiency is especially marked in the last sample and in the high calcium treatments; consequently the three interactions referred to above are all significant.

B. Ratio of fructosan to other sugars.

The significant effects and first order interactions of factors on the ratio of fructosan to other sugars are shown in Table VI. A comparison of this table

TABLE VI

Ratio of Fructosan to other Sugars: Significant Effects and First Order Interactions in Leaves and Stems

A. Mean effects of factors

	Potassium			Phosphorus			Sampling Date	
	Leaves.	Stems.		Leaves.	Stems.		Leaves.	Stems.
High	0.45	1.13	High	0.17	0.48	First sample	0.19	0.22
Medium	0.19	0.74	Low	0.34	0.90	Second sample	0.19	0.48
Low	0.13	0.20				Third sample	0.33	0.90

The mean effect of the balance of sodium and calcium is not significant.

B. First order interactions

I. Interaction of potassium with sodium-calcium balance (significant only for leaves).									
II. " of potassium with sampling date.									
III. " of phosphorus with sodium-calcium balance (significant only for leaves).									
IV. " of phosphorus with sampling date.									
				High potassium		Medium potassium		Low potassium	
				Leaves.	Stems.	Leaves.	Stems.	Leaves.	Stems.
I	{ High sodium	.	.	0.50	—	0.08	—	0.15	—
	{ High calcium	.	.	0.40	—	0.31	—	0.12	—
II	{ Second sample	.	.	0.29	0.70	0.12	0.64	0.15	0.15
	{ Third sample	.	.	0.61	1.56	0.27	0.84	0.12	0.24
				High phosphorus		Low phosphorus			
				Leaves.	Stems.	Leaves.	Stems.		
III	{ High sodium	.	.	0.21	—	0.27	—		
	{ High calcium	.	.	0.13	—	0.41	—		
IV	{ Second sample	.	.	0.14	0.18	0.23	0.77		
	{ Third sample	.	.	0.20	0.77	0.46	1.03		

with Tables IV and V shows that, in general, fructosan concentration and fructosan ratio are affected similarly by treatment. There are, however,

¹ H = high phosphorus; L = low phosphorus; K₁ = high potassium; K₃ = medium potassium (see Table I).

certain differences, the most important being with respect to the balance of sodium and calcium, the mean effect of which is significant for concentration only. The similarity of the effects of the other factors and of the more important interactions for the two types of data indicate that there is an inter-relationship between concentration and the partition of sugar between fructosan and other sugars. Further evidence for this is advanced below.

STATISTICAL ANALYSIS

The significance of treatment effects and interactions on the concentration of fructosan and the ratio of fructosans to other sugars was determined by the Analysis of Variance. The analysis is given in Table VII, values of *Z* being

TABLE VII

Analysis of Variance: Values of Z

Only significant values are included

	Degrees of freedom.	Concentration of fructosan.		Fructosan Other sugars		5 per cent.	1 per cent.
		Leaves.	Stems.	Leaves.	Stems.	point.	point.
Potassium . . .	2	1·7370	2·4280	1·5926	2·0266	0·6126	0·8626
Phosphorus . . .	1	1·9555	1·8540	1·4535	1·7751	0·7246	1·0285
Sodium-calcium balance	1	0·9605	1·1025	—	—	0·7246	1·0285
Sampling date . . .	1	1·6914	2·3941	1·2779	1·7711	0·7246	1·0285
Potassium/phosphorus	2	0·6528	—	—	—	0·6126	0·8626
Potassium/sodium-calcium balance	2	1·3908	1·3383	0·9208	—	0·6126	0·8626
Potassium/sampling date	2	1·8293	1·9903	0·9203	1·3090	0·6126	0·8626
Phosphorus/sodium-calcium balance	1	1·2167	1·1381	1·0485	—	0·7246	1·0285
Phosphorus/sampling date	1	1·0167	1·1640	0·7024	0·8258	0·7246	1·0285
Sodium-calcium balance/sampling date	1	—	—	—	—	—	—
Potassium/phosphorus/sodium-calcium balance	2	0·7477	—	—	—	0·6126	0·8626
Potassium/phosphorus/sampling date	2	—	—	—	—	—	—
Potassium/sodium-calcium balance/sampling date	2	0·8914	1·0752	0·6762	—	0·6126	0·8626
Phosphorus/sodium-calcium balance/sampling date	1	0·7823	1·0289	0·6348	1·7998	0·7246	1·0285
Potassium/phosphorus/sodium-calcium balance/sampling date	2	0·7078	—	—	—	0·6126	0·8626
Error . . .	24						
Total . . .	47						

shown only where they are significant. It should be pointed out that the analysis of variance is not rigidly applicable to the type of data here presented as there is a greater variability at the lowest potassium level than elsewhere. The method, however, is adequate for the present purpose.

To evaluate the effect of concentration on the partition of sugar between fructosan and other sugars the regression coefficients of fructosan ratio on total sugar have been calculated for both leaves and stems of the second and third samples (Table VIII). The regression lines are shown in Fig. 1. The regression coefficients are highly significant for the two stem samples and for the

TABLE VIII
Regression Coefficients of Ratio $\frac{\text{Fructosan}}{\text{Other Sugars}}$ against Total Sugar

b = regression coefficient.

	<i>b</i>	<i>t</i>	<i>p</i>
Leaves II (second sample)	0.068	0.70	>0.4
Leaves III (third ")	0.174	7.52	<0.01
Stems II (second ")	0.364	8.09	<0.01
Stems III (third ")	0.316	9.83	<0.01
Comparisons: Leaves II and leaves III		1.05	>0.3
Stems II and stems III		0.86	>0.4
Leaves II and stems II		2.73	<0.02
Leaves III and stems III		3.63	<0.01

later leaf sample (Table VIII). There is no significant difference between the regression coefficients of the two leaf samples or the two stem samples, but there are significant differences between the regression coefficients for leaves and stems on both occasions.

The analysis of covariance has been employed to determine whether the concentration of total sugar is the sole factor determining the ratio of fructosan to other sugars. The analysis of covariance enables the effect of the independent variate (total sugar in this case) to be eliminated so that the specific effect of treatment on the dependent variate (the ratio of fructosan to other sugars) may be evaluated. The analysis is given in brief in Table IX. Manurial treatment has no significant direct effect on the partition of sugar in stems, though the effects of sodium-calcium balance and phosphorus fall only slightly short of the level of significance. In the leaf sample, on the other hand, the direct effects of both potassium and sodium-calcium balance are significant.

DISCUSSION

The investigation described in this paper is part of a coordinated series of investigations of the effects of manurial nutrition on the growth and metabolism of the barley plant. The effects of a wide range of nutrient deficiencies have been studied, the manurial treatments employed in the present investigation being selected to show marked effects on growth and metabolism. For a comprehensive survey of the results at present available reference should be made to the recent review of Gregory (1937). The scope of the present communication is limited to a consideration of the conditions favouring the formation of fructosan, but as it is impossible to discuss one metabolic process

without reference to other closely related processes frequent reference will be made to the results cited in the review and elsewhere.

The level of sugar within the plant is determined by the balance between the rates of carbon assimilation and the rates of the various metabolic processes

TABLE IX

Analysis of Covariance: Ratio $\frac{\text{Fructosan}}{\text{Other Sugars}}$ and Concentration of Total Sugar

The values for mean square and Z are included only where this effect is significant or nearly so.

	Degrees of Freedom.	Leaves.				Stems.				5 per cent. point.
		Sum of squares.	Corrected mean square.	Z.	Sum of squares.	Corrected mean square.	Z.			
Potassium .	2	0.452905	0.2251	1.351	0.181015	—	—	0.615		
Phosphorus .	1	0.033309	—	—	0.505634	0.19638	0.696	0.729		
Sodium-cal- cium balance	1	0.301100	0.0658	0.738	0.386990	0.13745	0.566	0.729		
Sampling date	1	0.019271	—	—	0.002431	—	—	0.729		
Error .	23	0.345290	0.0138	—	1.123237	0.048836	—	—		

Sum of squares calculated as $\sum (y - bx)^2$, where $\sum y^2$ = sum of squares for fructosan ratio, $\sum x^2$ = sum of squares for total sugar, and b = regression coefficient. Mean square corrected by the method of Yates (1934).

in which carbohydrate is utilized. This is clearly illustrated in the growth of the barley plant, in which the minimal concentration of sugar occurs when the rate of meristematic growth (i.e. tillering) is maximal (Gregory and Baptiste, 1936; Archbold, 1938a). Subsequently meristematic activity declines and sugar concentration rises (i.e. third sample). At a later stage polysaccharide synthesis is associated with the elongation of the stem and the development of the ear and the sugar content of the plant again declines (Archbold, 1938a; Barnell, 1936). The accumulation of sugars gives therefore no evidence of increased assimilation or of the hydrolysis of previously formed carbohydrates, but shows rather that the production of sugar by assimilation is temporarily in excess of the requirements of the various metabolic processes in which carbohydrate is utilized, namely respiration and meristematic activity with the associated processes of protein and polysaccharide synthesis. In the light of these considerations the known effects of manurial deficiency on the level of fructosan and other sugars will be examined briefly.

Potassium deficiency. There is considerable evidence both in the results here presented and in earlier work (Shih, 1936; Russell, 1937) that the effects of potassium deficiency in the presence of high sodium differ markedly from those developed in the presence of high calcium. *Potassium deficiency in the presence of high sodium* causes a marked reduction in the level of all sugar fractions (Gregory and Baptiste, 1936; Gregory and Sen, 1937). It has been shown that these plants are characterized by a greatly reduced assimilation

rate, and a considerable increase in respiration rate and tiller production (i.e. meristematic activity) (see Gregory, 1937, for references). As a result of these effects the concentration of soluble carbohydrates is low. *Potassium deficiency in the presence of high calcium* results in an increased sugar concentration in the early leaves and in a decreased concentration in the later leaves (Janssen and Bartholomew, 1930; Russell, 1937). The initial increase due to this type of potassium deficiency results from a marked curtailment of vegetative growth associated with an assimilation rate only slightly lowered. At a later stage the assimilation rate of the potassium deficient treatments decreases very rapidly and translocation of sugar to the roots is high, as shown by the increased ratio of roots to tops (Shih, 1936); thus the concentration of sugar is lowered, though not as markedly as in the high sodium treatments. The data of the present investigation show that fructosan content is affected by potassium deficiency even more markedly than is total sugar. Not only the concentration of fructosan but also the ratio of fructosan to other sugar is lowered. This effect is greater in the high sodium than in the high calcium treatments.

Phosphorus deficiency. Vegetative growth is greatly restricted by phosphorus deficiency and at the level used assimilation is unaffected (see Gregory, 1937). These treatments are characterized by a high level of soluble carbohydrates. The present data show that in general the accumulation of fructosan is particularly marked, the ratio of fructosan to total sugar being much increased. Further effects of phosphorus deficiency are discussed by Richards (1938).

Nitrogen deficiency. In nitrogen-deficient plants protein synthesis is much reduced and vegetative growth is consequently curtailed. Assimilation rate is unaffected and carbohydrates again accumulate. Archbold's (1938a) results show that the carbohydrate accumulated under these conditions is largely fructosan.

This survey of the effects of manurial deficiency on fructosan content indicates clearly that treatments which cause a supernormal concentration of sugar result in an increase of the ratios of fructosan to other sugars.

Archbold (1938a) has found that the sugar accumulation in leaves and stems resulting from the removal of ears is also associated with the formation of a high proportion of fructosan. This accumulated sugar represents material which, for the time being, is not utilized in metabolism and it was therefore suggested by Archbold that there is a tendency for temporarily immobilized carbohydrate to be converted to fructosan.

In Fig. 1 the ratio of fructosan to other sugars is plotted against the concentration of total sugar. Regression coefficients have been calculated and, except for the earlier leaf sample, they are very highly significant (Table VIII). This shows clearly that the partition of soluble carbohydrate between fructosan and other sugars is largely, if not entirely, controlled by concentration. The statistical methods described on p. 874 have enabled the independent effects of concentration and manurial treatment on the ratios of fructosan to other sugars to be determined. It is shown (Table IX) that in stems there is no significant *direct* effect of manurial treatment, so that sugar concentration

is the sole factor which determines the ratio of fructosan to other sugars in stems. In leaves, on the other hand, significant effects of the sodium-calcium balance and of potassium are found. Thus in leaves we may distinguish two types of effect of manurial treatment on the ratios of fructosan to other sugars, namely, a *direct* effect, and an *indirect* effect resulting from the changes in sugar concentration. An examination of the data discloses that the *direct* effect of potassium deficiency is to lower the fructosan ratio and that the ratio is higher with high sodium than with high calcium for the same concentration of total sugar. While it is not possible fully to explain these direct effects the following considerations are of interest. The respiration of leaves is known to be greatly increased by potassium deficiency (see Gregory, 1937, for references), and it is possible (Onslow, 1931) that fructose is preferentially respired. This should lead to a reduced ratio of total fructose to total glucose in the potassium deficient treatments and as a result the ratio of fructosan to other sugars would be lowered. As in stems the rate of respiration is much lower than in leaves the effect of potassium would be expected to be found in leaves only, and this has been shown to be the case. The role of the balance of sodium and calcium in determining the partition of sugar is obscure. In the present investigation the effect is significant in leaves only, but falls only slightly short of the level of significance in stems, so that it is possible that a more sensitive technique would reveal a significant effect there also.

It will be noted that in both leaves and stems sampling date has no direct effect on the ratio of fructosan to other sugars (see Tables VIII, IX). The increase in ratio of fructosan to other sugars between the second and third samples (Table VI) is thus proved to be due solely to increase in concentration of total sugar. For any given level of sugar it thus appears that in both leaves and stems a constant and characteristic ratio is found between fructosan and other soluble sugars. The significant difference between the values for the regression coefficient of stems and leaves in both samples (Table VII) shows that for a given level of total sugar a higher ratio of fructosan to other sugars obtains in stems than in leaves. This is probably due to the fact that the rapid translocation of sugars away from the leaves prevents fructosan, a secondary product, from reaching a high level in the leaf. Fig. 1 shows that the regression lines do not pass through the origin. Except in the first leaf sample where the regression coefficient is not significant, the regression lines cut the abscissa at points corresponding to a total sugar concentration of about 0.5 per cent., indicating that when the concentration of total sugar is below this level no fructosan is formed.

The above considerations show that within the limits of concentration here observed the linear relationship is an adequate expression of the balance of carbohydrate. The comparatively simple effect of manurial treatment on the partition of total sugar is in marked contrast with the complex interactions which affect concentration, and suggests that stored sugars tend to equilibria controlled by factors, presumably enzymatic, which are relatively unaffected

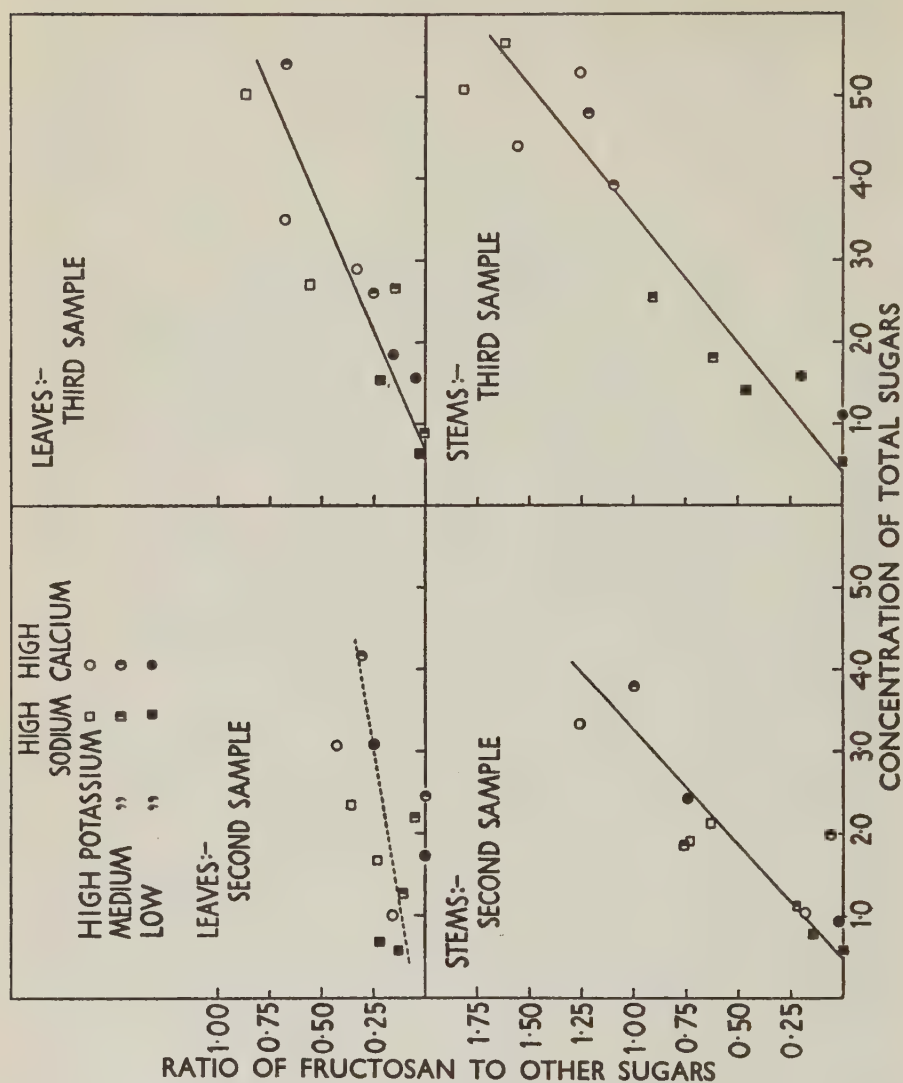


FIG. 1. Regression lines of the ratio $\frac{\text{fructosan}}{\text{other sugars}}$ against total sugar in leaves and stems of the barley plant. The points shown are means of duplicate determinations.

by manurial deficiency. On the other hand, the concentration of sugar is determined by the balance of metabolic processes which show a very marked response to manurial treatment.

SUMMARY

Barley plants were grown in sand culture under twelve manurial treatments, comprising various levels of potassium, phosphorus, and the balance of sodium and calcium.

Separate extracts of fructosan and the other water-soluble carbohydrates in leaves and stems were made on three occasions during the development of the plant.

To facilitate the extraction of stems prior to analysis a grinding mill was devised, a description of which is given (see appendix).

The effects and interactions of manurial treatment on fructosan content are discussed; it is shown that the concentration of fructosan is lowered by potassium deficiency, especially in the high sodium treatments. Deficiency of phosphorus increases the level of fructosan.

The ratio of fructosan to other sugars is in general affected by treatment in the same way as is the concentration of fructosan.

Statistical analyses show that in stems this ratio is entirely dependent on the concentration of total sugar, the ratio being high when the concentration of total sugar is high. In leaves a similar relationship holds, but in addition potassium deficiency and the balance between sodium and calcium show effects independent of concentration charges.

For a given concentration of total sugar the fructosan ratio is higher in stems than in leaves. This, it is suggested, may be a result of rapid translocation from the leaves.

The relationship between concentration of fructosan and its ratio to other sugars appears to be independent of the age of the plant in both leaves and stem during the period of vegetative development.

The results here presented are compatible with the view that surplus sugar is temporarily stored as fructosan.

This investigation was undertaken at the suggestion of Professor F. G. Gregory, to whom the writer is deeply indebted for constant advice and stimulating encouragement. He desires to express thanks also to Dr. H. K. Archbold for advice on analytical methods, to Mr. F. J. Richards for advice on field experimental methods, and to Mr. W. E. Montgomery for assistance with the analytical work.

APPENDIX

A Grinding Mill for Quantitative Work

A description of the mill constructed for grinding barley stems is given here, as the instrument should be of use in other processes when it is necessary to grind fresh or wet material without loss of leaves or fluid. The principle

employed is a modification of that used by Dutcher and Laudig (1924) for soft tissue. The apparatus here described is specially suited for treating lignified or other hard material.

The construction of the mill is shown in the accompanying diagram and photographs (Figs. 2-4). The material is ground in the narrow space between a rotating cone (*a*) (see Fig. 2), and its outer casing (*d*) which remains stationary.

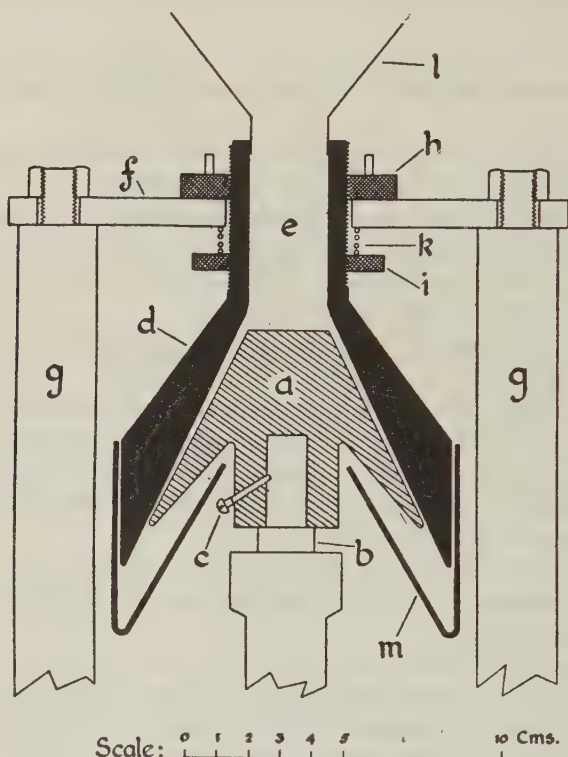


FIG. 2. Diagrammatic section of grinding mill. For details see text.

Both are solidly built of gun-metal. The cone (*a*) is secured by a lock screw (*c*) to a spindle (*b*) which is rotated at the speed of 50-60 r.p.m. by a small electric motor with gear-box and chain drive. The hollow stem (*e*) of the outer casing (*d*) is mounted on an iron plate (*f*) on which it is prevented from rotating by means of a key and key-way. Vertical adjustment is possible by turning the large nuts (*h*) and (*i*), whereby the distance between the two grinding surfaces may be altered. A strong spring (*k*) provides a slight degree of play which ensures the smooth running of the mill. Four rigid bronze pillars (*g*) support the plate (*f*). The mill is fed through the hollow stem (*e*) of the outer casing, a detachable funnel (*l*) being employed.

Both grinding surfaces are provided with sharp-edged grooves which constitute two orthogonal spiral systems (see Fig. 4). The cone is scored with

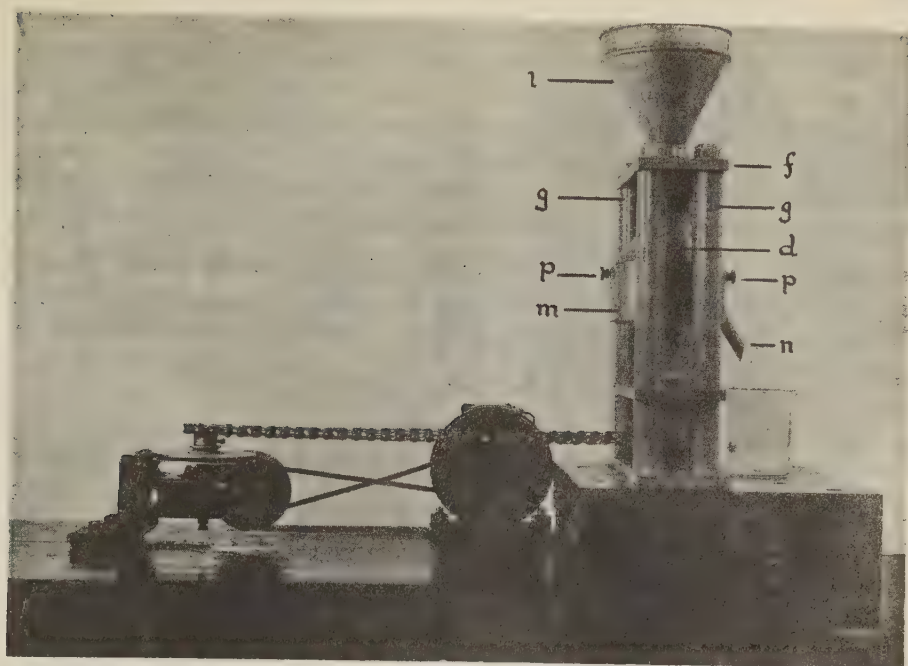


FIG. 3. General view of grinding mill. For details see text.

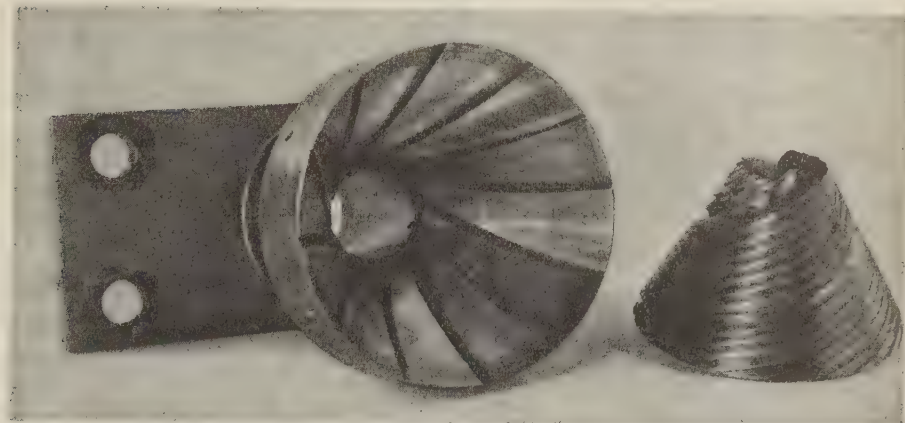


FIG. 4. Grinding mill: The grinding cone and its outer casing, showing sharp-edged cutting grooves.

two principal spirals which taper downwards and provide at the top two large openings into which the coarsely cut stems enter readily. Once engaged in the spirals, the material is drawn down by friction and is disintegrated by the action of the opposed systems of sharp-edged grooves. The fine spirals of the cone complete the pulping and the material emerges in a finely shredded form into an annular receiver (*m*) (see Figs. 2 and 3), which is secured to the

outer casing of the mill by two screws (*p . . . p*) (Fig. 3). An outlet (*n*) permits fluid and the finer fragments to pass out. It is convenient to employ a metal plunger to press the material down the stem (*e*) of the outer casing until it engages with the spirals of the cone. After use the mill is washed with small amounts of water while the cone is still rotating. The mill is then dismantled and the receiver is emptied. A 50 gm. sample can be dealt with in five minutes; for larger samples a bigger receiver with a wider outlet would be an advantage.

The writer is indebted to Mr. W. Shaw, technician to the Botany Department, Imperial College, for much valuable assistance, and to the Unicam Instrument Company, Cambridge, who constructed the greater part of the mill.

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The Size of the Chloroplasts in Eupolyploid Forms of *Nicotiana* and *Solanum*

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EUPOLYPLOIDY in plants conditions a series of heritable changes. Some are 'directed', others are not. Directed, heritable variations in plants which show an increase with the euploid increase of the chromosomes are: (1) the amount of the nucleolar substances (number of nucleoli, size, or both), (2) the size of the nuclei, (3) the amount of cytoplasm, (4) the volume of the cells, (5) the breadth of the leaves, and (6) the size of the seeds or of grains (Kostoff, 1938). With the euploid increase of the chromosomes the vegetation period (the period between sowing and flowering) is usually prolonged. Characters like the size of the plants and the size of the flowers are also influenced by the euploid chromosome alterations, but there is no correlation between euploid chromosome alteration and the expression of these characters. In some cases the size of the plant increases with the euploid increase of the chromosomes, in other cases it decreases. The size of the flowers (corolla, calyx, &c.) vary in a similar way (Kostoff, 1938).

The chloroplasts seem to behave differently from the characters mentioned above. This is at least true for the higher plants, especially for *Nicotiana* and *Solanum* polyploids which we have studied.

According to the observations reported by Gerassimow (1902), the spiral chloroplast ribbons of *Spirogyra* cells with two nuclei or with increased nuclei (probably polyploid) became 'um die Kerne herum breiter, stärker geschlängelt, mit einem mehr lappigen Rand, an den Enden der Zellen aber sind sie schmaler, und ihr Rand ist einfacher. Daraus folgt mit Sicherheit, dass der Kern einen Einfluss auch auf die Entwicklung der Chlorophyllbänder ausübt' (p. 248). Even after a year cells with enlarged nuclei had a larger number of chlorophyll bands. The average number of the chlorophyll bands in normal cells were eight, while in the cells with larger nuclei they have been on the average twelve, and in the binucleate cells on the average thirteen. Similar conditions have been found in *Zygnema* (Gerassimow, 1905, p. 51). Winkler (1916) studied the size of the chloroplasts in diploid and tetraploid *Solanum nigrum* and *S. Lycopersicum*. Exact measurements

were not carried out, but he drew a few chloroplasts (pp. 460, 465) from each form and made the following generalization: 'Die Chlorophyllkörner und auch die Leukoplasten sind nämlich in den Zellen der Gigasform grösser als in entsprechenden Zellen der Stammform.' This was only a general impression as he mentions later in his paper. The statement made by Winkler (1916) has been referred to in some more recent investigations. In the cases where data have been found contradictory, very cautious conclusions have been drawn. Schweizer (1923), for example, studied the chloroplasts in haploid and diploid *Splachnum sphaericum* and concluded: 'Merkwürdigerweise erreichen die Chloroplasten knapp die Grösse derjenigen des haploiden Protonemas' (p. 40). Consequently he refused to use them as an indicator of eupolyploidy. Wettstein (1923, 1925) studied the size of the chloroplast with the euploid increase of the chromosome numbers in mosses and stated: 'Schliesslich wird die Zahl der Chloroplasten erhöht, ihre Grösse bleibt aber nach bisherigen Beobachtungen unverändert.' And further: 'In diesem Punkte stehen die Moose in einem gewissen Gegensatz zu plurivalenten Blütenpflanzen wie *Solanum* nach den Beobachtungen von Winkler 1916' (p. 21).

Kostoff and Kendall (1934) studied the size of the chloroplasts in a tetraploid tomato and their measurements showed 'that the chloroplasts are not larger in the tetraploid plants; if any difference, there is a tendency to a smaller size than in the diploids. Strangely enough, then, the results agree with those obtained in the mosses, but disagree with those obtained by Winkler in another race of the same species of plant (*Solanum Lycopersicum*)' (p. 34).

Considering the existing discrepancies upon this question we started this work on a large scale, investigating a series of experimentally produced autopolyploid and allopolyploid plants and parental species of the allopolyploids, some of which, as *Nicotiana rustica*, represent natural allopolyploids. Polyploid forms, measured, were experimentally obtained in our laboratory (Kostoff, 1935, 1938; Kostoff and Kendall, 1934). Diploid and autotetraploid *N. glauca* were kindly supplied by G. D. Pratesenia, for which we express here our gratitude. The plants studied are shown in the table.

Young but completely developed leaves were taken from the plants and fixed in 80 per cent. alcohol on August 3, 1937. Such leaves were chosen on the view of the late V. N. Lubimenko that in very young leaves which are not completely developed the chloroplasts have not reached their full size; in the old leaves, on the contrary, they degenerate.

The material fixed was studied in winter and early spring. The leaves were first transferred to water. After one to two hours small blocks were taken from various parts of the leaves, were crushed on the object glass in water, then covered and examined under the microscope. The measurements were made with an ocular micrometer with high magnification (ocular 12 compens. and oil immersion 1/12) in order to limit the possible errors (one division of the ocular micrometer corresponded to 1 μ).

The data obtained are given in the table. They show that chloroplasts do not increase in size with the increase of the chromosome number. On the contrary, in all cases there is a tendency to smaller size than in the diploids or in the parental species. The differences between the diameter of the plastids in diploid and tetraploid *N. glauca* are significant, diploids having larger plastids than tetraploids ($M_d - M_t = 1.18 \pm 0.075$).

No.	Plants.	Somatic chromo- some no.	Diameter (μ) of the chloroplasts.							n	M	σ
			2.5	3.5	4.5	5.5	6.5	7.5	8.5			
1	<i>Solanum Lycopersicum</i> (diploid)	24	—	130	256	104	10	—	—	500	4.48	0.90
2	<i>Solanum Lycopersicum</i> (tetraploid)	48	4	158	247	88	3	—	—	500	4.35	1.35
3	<i>Nicotiana glauca</i> (diploid)	24	—	—	39	129	62	18	2	250	5.76	0.84
4	<i>Nicotiana glauca</i> (tetraploid)	48	—	22	98	26	4	—	—	150	4.58	0.64
5	<i>Nicotiana rustica</i> var. <i>texana</i> .	48	—	—	38	56	6	—	—	100	5.18	0.59
6	Amphidiploid (<i>Nicotiana rustica tex-</i> <i>ana</i> $\times$ <i>N. glauca</i> .	72	—	5	37	51	7	—	—	100	5.10	0.70
7	<i>Nicotiana suaveolens</i> .	32	—	2	23	55	18	2	—	100	5.45	0.82
8	<i>Nicotiana multivalvis</i> .	48	—	14	57	26	3	—	—	100	4.68	0.70
9	Amphidiploid (<i>N. multivalvis</i> $\times$ <i>N.</i> <i>suaveolens</i>) .	80	—	14	47	34	5	—	—	100	4.80	0.77
10	F ₁ <i>N. multivalvis</i> $\times$ <i>N.</i> <i>suaveolens</i> .	40	—	14	30	44	12	—	—	100	5.04	0.88
11	<i>Nicotiana rustica hu-</i> <i>milis</i> .	48	—	4	38	48	10	—	—	100	5.14	0.70
12	<i>Nicotiana paniculata</i> .	24	—	3	29	56	18	—	—	100	5.27	0.70
13	Amphidiploid (<i>Nicotiana rustica hu-</i> <i>milis</i> $\times$ <i>paniculata</i>) .	72	—	3	20	52	23	2	—	100	5.51	0.79
14	Amphidiploid (<i>N. rustica</i> $\times$ <i>N. taba-</i> <i>cum</i> var. <i>Basma</i>) .	96	2	24	55	18	1	—	—	100	4.42	0.86
15	<i>N. tabacum</i> var. <i>Basma</i>	48	—	10	39	41	9	1	—	100	5.02	0.82
16	Amphidiploid (<i>N. rustica</i> $\times$ <i>N. taba-</i> <i>cum</i>) $\times$ amph. (<i>N.</i> <i>multivalvis</i> $\times$ <i>suaveo-</i> <i>lens</i>) .	88	—	13	42	41	4	—	—	100	4.86	0.84
17	<i>Nicotiana Langsdorffii</i>	18	—	18	55	26	1	—	—	100	4.60	0.69
18	Amphidiploid (<i>N. glauca</i> $\times$ <i>N. Langs-</i> <i>dorffii</i> .	42	—	10	55	32	3	—	—	100	4.78	0.68

The average diameter of the chloroplasts in the amphidiploid *N. rustica* $\times$ *paniculata* is the only one that is somewhat larger than the average diameter of the parental species. The difference, however, in respect to *N. rustica* is on the limit of significance (0.37 ± 0.1), while in respect to *N. paniculata* the difference is non-significant (0.24 ± 0.1).

Since tetraploids have usually a deeper green colour but not larger chloroplasts, the differences in colour should result from the larger number of

chloroplasts in the cells. We have not made exact counts in *Nicotiana* polyploids, but some counts made in diploid and tetraploid tomatoes showed that this conclusion is correct. It is difficult to determine the kind of causal relation existing between the number of the chloroplasts and the tendency stated above, that the diameter of the chloroplasts decreases with the duplication of the chromosome number. Present studies suggest, however, a great autonomy of the chloroplasts in respect to the euploid chromosome alterations.

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Studies on the Nitrogen Metabolism of Plants

III. On the Effect of Water Content on the Relationship between Proteins and Amino-Acids

BY

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With three Figures in the Text

NOTATION:

P , protein-nitrogen content on a dry-weight basis.

$[P]$, concentration of protein-nitrogen.

A , amino-nitrogen content on a dry-weight basis in general.

A_T , total amino-nitrogen content on a dry-weight basis.

A_R , residual amino-nitrogen (A_T minus amino-N of asparagin) on a dry-weight basis.

$[A]$, $[A_T]$, concentration of amino-nitrogen.

U , water content on a dry-weight basis.

V , defined in Table II.

INTRODUCTION

IN the first paper of this series (Petrie and Wood, 1938) it was shown that, under conditions of constant illumination and temperature, the amount of protein in the leaves of plants increases both with the amino-acid content and with the water content; and, although under such conditions the system did not attain a constant steady state, and may not even have attained a drifting steady state, it was considered probable that such states would be characterized by the same relationship. It was pointed out that there are two ways in which water content may produce an effect on the relation between the amounts, expressed on a dry-weight basis, of protein and amino-acids: firstly, the effect may be merely the result of changes in the concentrations of these nitrogen compounds; or secondly, the change in water content may specifically alter the rate of one or more reactions in the system.

In considering the first of these ways it was pointed out that the results

¹ This investigation is one of a series financed co-operatively by the Carnegie Corporation of New York, the Australian Council for Scientific and Industrial Research, and the University of Adelaide.

could be explained if, with increase of concentration of proteins and amino-acids from one pair of steady state values to another,

$$\frac{\Delta[P]}{\Delta[A]} < \frac{[P]}{[A]}.$$

If this inequality holds, then a reduction of water content would cause protein hydrolysis until a new steady state is established. This inequality could exist if the $[P]$ - $[A]$ curve is concave to the $[A]$ axis, or if it is convex over a limited range of the variables.

If, on the contrary,

$$\frac{\Delta[P]}{\Delta[A]} > \frac{[P]}{[A]},$$

a reduction in water content would result in a quantity of protein being synthesized before the new steady state is attained. This inequality could occur if the $[P]$ - $[A]$ curve were convex to the $[A]$ axis.

Finally, if

$$\frac{\Delta[P]}{\Delta[A]} = \frac{[P]}{[A]},$$

then

$$[P] = k[A],$$

and change in water content would have no effect on the amounts of proteins and amino-acids in the leaves.

Any of these phenomena might, of course, be superimposed on a specific effect of water content on the rates of synthesis or hydrolysis. Furthermore, these possibilities are formulated on the hypothesis that protein synthesis occurs by the linking together of amino-acids: if this hypothesis is incorrect, the possibilities would require to be expressed differently; however, we have not attempted such different expression at the present time.

In this paper it is proposed to consider these possibilities further, in an endeavour to obtain indications as to which of them is likely to be the true state of affairs. Additional data upon which to base our consideration are also provided by the description of some experimental work other than that already presented.

EXPERIMENTAL

Experiment III.

This experiment has been described in paper I. However, it was also used for an investigation of sulphur metabolism by J. G. Wood and B. S. Barrien, the results of which are not yet published. During the course of this investigation, free cystine was estimated in the same filtrate as that used for the estimation of the soluble-nitrogen fractions. The methods used were Lugg's modification of the Folin-Marenzi method (Lugg, 1932), and Lugg's modification of Sullivan's method (Lugg, 1933); the two methods gave closely agreeing results and means for duplicate estimations by both were taken for the final values. No cysteine or glutathione could be detected in the filtrates.

We shall have occasion in this paper to refer to these cystine data, and they are therefore presented graphically in Fig. 3.

Experiment IV.

The procedure of this experiment was similar to that described for experiments I-III in paper I. Seeds of *Lolium multiflorum* Lam. were sown on September 29, 1934, in glass jars containing 3.75 kg. water-washed sand maintained with distilled water at 60 per cent. of its saturation capacity. The culture solutions used were the same as in the previous experiments. All pots were placed in the cabinets on November 13, 1934 (day 0), and removed for analysis on November 15 (day 2) and 17 (day 4). Analyses were also performed on plants taken from two pots selected at random on November 13 at the time of transference of the other pots to the cabinets.¹

The following treatments were applied twenty-four hours after the pots had been placed in the cabinets:

C	.	.	.	0	gm. (NH ₄) ₂ SO ₄ per pot			
N ₁	.	.	.	0.15	"	"	"	
N ₂	.	.	.	0.45	"	"	"	
N ₃	.	.	.	0.9	"	"	"	

The water content of the sand was also raised in certain pots and allowed to fall in others after they were placed in the cabinet, the final values being 70, 40, 25, and 15 per cent. of saturation; these are hereafter indicated by the figures 1-4 subscribed to the treatment designations: the last two levels were probably not attained until after day 2, since weighings of the pots on the evening of day 1 showed that the C₃ pots still had a water content of 40 per cent., and the C₄ pots one of 27 per cent. of saturation.

Two pots per treatment were used for each analysis. The results are given in Table I and Figs. 1 and 2.

DISCUSSION

General consideration of results of experiment IV.

Changes with time and treatment. This experiment differed from those previously reported by including data from a sample of the population of plants at the time they were placed in the cabinets. In every case, placing the plants in the cabinets has caused an initial rise in *U*, probably because of reduced transpiration rate. This initial rise is greater in successive members of the series C₁-C₄; in C₃ and C₄² there is a subsequent fall between days 2 and 4, by which time the external supply of water had become greatly reduced.

In cases where no extra nitrogen was supplied there is a marked initial fall in *P*; but in general this fall is less in successive members of the series C₁-C₄.

¹ Hence the change in our convention of numbering the days of the experiment.

² These designations have been transferred at will from the treatments to the plants throughout the paper.

On the contrary, where extra nitrogen was supplied, P rises except in N_{I_3} . The drifts in total nitrogen reflect those in protein nitrogen.

There is no appreciable drift in A_T except where extra nitrogen was supplied. Where the record extends to day 4 it is suggested that a steady state

EXPERIMENT IV

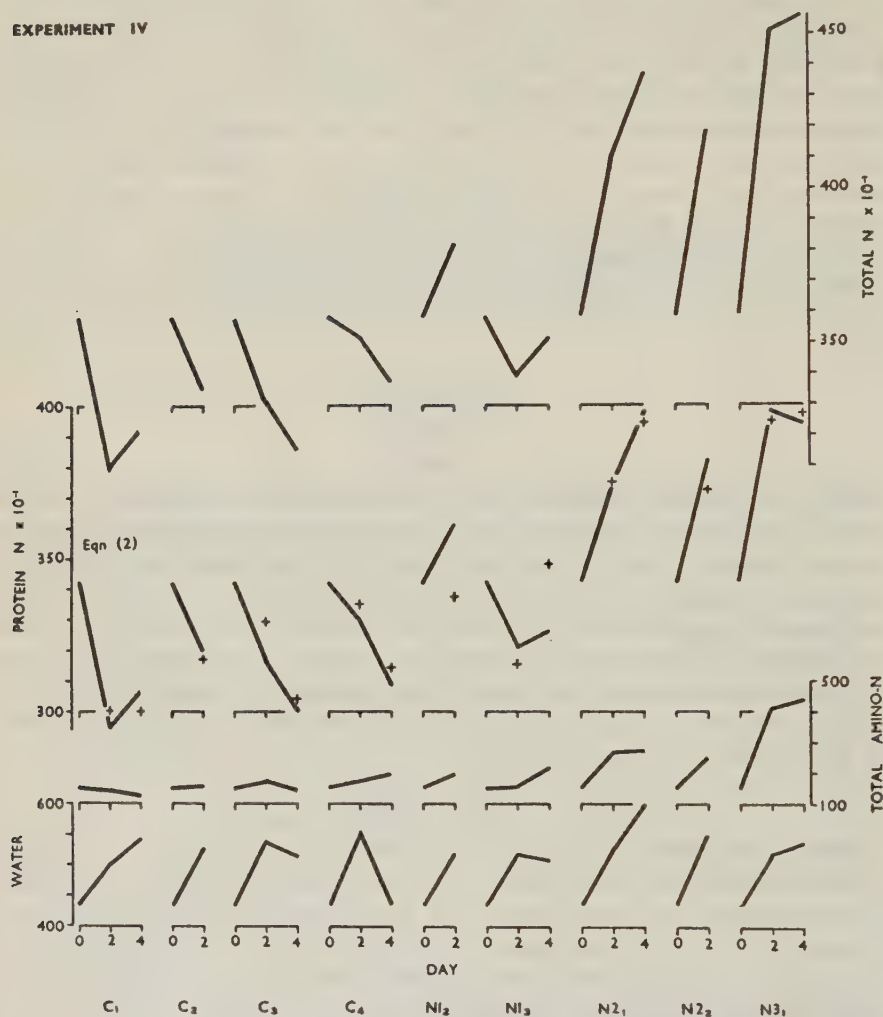


FIG. 1. Drifts with time in the contents of various components of the leaves in experiment IV. Values of P calculated from equation (2) of Table II are also plotted. (From data of Table I, wherein the units are defined.)

has been reached on day 2, as no further appreciable change in A_T takes place. The large amount of protein hydrolysed between days 0 and 2 never accumulates in the leaves as amino-acids: evidently translocation is sufficiently rapid to keep pace with any appreciable amount of soluble nitrogen compounds formed above that characterizing the steady state.

TABLE I
Results of Experiment IV

DAY 0										
Total amino-N										148
Prot. N $\times 10^{-1}$										342
Total N $\times 10^{-1}$										359
Water										435
Treatment.										
DAY 2										
	C ₁ .	C ₂ .	C ₃ .	C ₄ .	NI ₁ .	NI ₂ .	N ₂₁ .	N ₂₂ .	N ₃₁ .	
Total amino-N		141	156	171	173	194	157	268	248	410
Prot. N $\times 10^{-1}$		295	319	316	330	361	321	374	382	398
Total N $\times 10^{-1}$		310	336	332	352	383	340	411	419	451
Water		500	525	536	552	517	517	529	550	517
DAY 4										
Total amino-N		125	—	141	193	—	219	270	—	439
Prot. N $\times 10^{-1}$		307	—	300	309	—	326	398	—	394
Total N $\times 10^{-1}$		323	—	316	338	—	352	438	—	456
Water		540	—	513	437	—	508	598	—	532

Nitrogen fractions in mgm. per 100 gm. dry weight.

Water in gm. per 100 gm. dry weight.

In general it is apparent that, after day 2, the P drifts can be interpreted on the assumption that P increases with both A_T and U .

Regression functions. The regression of P on A_T and U is given in Table II. There is evidence that $\partial^2 P / \partial A_T^2$ is negative, since the coefficient of the term in A_T^2 is significant and negative. The regression accounts for 87 per cent. of the variance of P , and the calculated values plotted in Fig. 1 show that the agreement between these and the observed values is on the whole good. The curve of the regression equation is also plotted in Fig. 2, where the data for days 2 and 4 are collected together.

The concavity of the P - A_T curve. With four exceptions, the values of U lie between 500 and 540. Neglecting the regression curve, it is possible in Fig. 2 to pick out the points corresponding to these closely agreeing values of U ; it is then seen that, with such approximately constant U , there is considerable evidence that the P - A_T curve is concave to the A_T axis.

An explanation for the concavity might be that, with high A_T , P was at the times of observation considerably lower than the steady state value; on the other hand, with low A_T a drifting steady state might have been attained. But, in the case where A_T has its highest value, P is no longer rising; in fact the curves for N₃₁, Fig. 1, suggest that between days 2 and 4 the system remained fairly close to a steady state. The two points on the right of Fig. 2 ($U = 517$ and $U = 532$) cannot therefore be regarded as having values of P below those characterizing the steady state. The curvature could also have been produced by points $U = 598$, $U = 550$, and $U = 529$ having P values above those of the steady state; but Fig. 1 shows that the first and last of these points are associated with rising P , so that P would tend to be at or

below the steady state value; and the value $U = 550$, although associated with falling P , is lower than the value calculated from the regression based on all the data combined.

Approach to steady state. These arguments rest on the assumption that, when the drift in U or A_T changes in direction, the lag in P is not great. On the whole the data support this assumption, as did those of the previously reported experiments. Thus, although from day 0 the tendency is for P to fall rapidly, this tendency was reversed where appreciable amounts of extra nitrogen were supplied. While the whole system is drifting between days

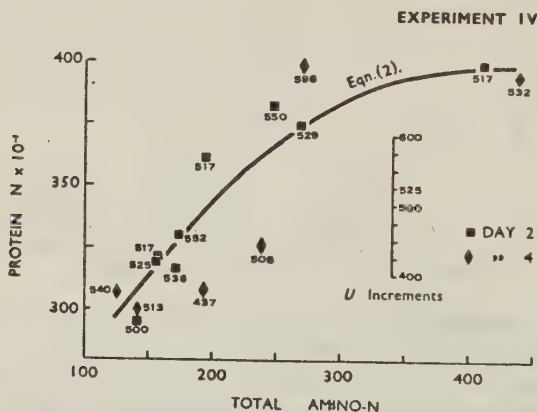


FIG. 2. P plotted against A_T , experiment IV. The corresponding value of U is given for each point. The curve is that of equation (2), Table I, for the mean value of $U (= 525)$. The scale of U increments shows the distance that, according to the equation, has to be added to or subtracted from the ordinate of any point on the curve to give the value of P corresponding to a point with the same value of A_R but with a value of U above or below 525. Units are as given in Table I.

2 and 4, there is, therefore, evidence that it was in most cases not greatly removed from a drifting steady state; this experiment thus adds strength to the view that, at the steady state, $\partial^2 P / \partial A_T^2$ is negative.

It is clear that the P - A_T - U relation on day 0, pertaining to the environment of the glass-house, was very different from that pertaining to the conditions in the cabinets: from equation (2), $P_{\text{calc.}} \times 10^{-1}$ for day 0 is 287, $P_{\text{obs.}} \times 10^{-1}$ being 342. Either the system was far from a steady state on day 0, on account of rapid environmental changes, or else some other factor determining P , such as temperature or light, which was constant and different under the conditions of the cabinets, caused the high value.

The rise in water content with falling external supply. A curious feature of the data is that U rises from C_1 to C_4 on day 2, whereas the water content of the sand falls. One result of this fall would be that the concentration of ammonium ions in the external solution was increased: in the case of C_4 it must have been at least $2\frac{1}{2}$ times that in C_1 on day 2. This may be the cause of the rise in total- and protein-nitrogen content from C_1 to C_4 .

Now it has frequently been found that increase in protein and total-nitrogen content increases the water content of leaves. This was actually suggested in the previous experiments (paper I) where, with the first increase in nitrogen supply, there was frequently an increase in both *P* and *U*. It has

TABLE II
Regression Equations

Both regressions are significant at the 1 per cent. point. All coefficients are significant at or below the 5 per cent. point.

NOTATION

V = percentage of the variance of the dependent variable ascribable to the average effect of the independent variables; for derivation see Table I, paper II (Wood & Petrie, 1938).¹

D.F. = residual degrees of freedom.

$\sqrt{\text{res. var.}}$ = square root of variance of dependent variable not accounted for by the regression.

The remaining symbols are as given at the commencement of the paper.

The units are as given in Table I.

EXPERIMENT IV.

	<i>V</i>	D.F.	$\sqrt{\text{res. var.}}$
(1) $P = a + b_1 A_T + b_2 U$	82	12	162
$a = 674$			
$b_1 = 3.18 \pm 0.464$			
$b_2 = 3.90 \pm 1.277$			
(2) $P = a + b_1 A_T - b_2 A_T^2 + b_3 U$	87	11	136
$a = 389$			
$b_1 = 10.4 \pm 2.51$			
$b_2 = 0.0129 \pm 0.00440$			
$b_3 = 2.80 \pm 1.038$			

also been found by Crist (1926), Pearsall and Ewing (1929), Petrie (1937), Williams (1938), and others; Crist in addition found an increase in bound-water content as determined by the cryoscopic method. The extra water may be held by imbibition in the additional amount of colloidal protein present, and by increased dissociation thereof (cf. Petrie, 1937); but it may also be held in the vacuole, especially in view of the fact that a greater osmotic pressure of the cell-sap has been found by Eaton (1927), Petrie (1932), Mothes (1932), and Böning and Böning-Seubert (1934) to be associated with increased nitrogen supply to plants.

A second relation may thus be superimposed on the *P-U* relation already discussed, as was in fact suggested in paper I. The extent of the superimposition cannot be determined at present. However, states of affairs can be visualized where increase in water content would not lead to further net protein synthesis. Thus, if the amount of protein in the leaves is increased, leading to a greater amount of water being held by imbibition, only extensive, and not intensive, properties of the system may be changed, in which case the increase in water content may not lead to further net protein synthesis. Again, increased salt content of the vacuoles would lead to greater osmotic pressure, but may produce no change in suction pressure at the steady state,

provided transpiration rate is unaltered; in this case the amount of water in the cytoplasm would remain the same, and there may be no change in protein content. These states of affairs differ from any in which the rate of transpiration rises or falls, producing change in turgor and suction pressure, and so in concentrations in the cytoplasm, and hence change in net rate of protein synthesis.

By day 4 the water content of the external medium has fallen so drastically in C_4 that U is decreased, and now the relation discussed first manifests itself and the fall in U leads to fall in P .

Theoretical consequences of concavity in the $[P]$ - $[A]$ curve on the data expressed on a dry-weight basis.

If the observed effect of U on the P - A relation is due solely to a concentration effect, a probable explanation, as stated in the Introduction, is that the $[P]$ - $[A]$ curve is concave to the $[A]$ axis. It is of interest to explore more fully the consequences of such a property on the relation between P , A , and U .¹

For $[P]$ and $[A]$ we can write P/u and A/u , where u is the amount of water (per 100 gm. dry matter) present in the intracellular phase wherein protein synthesis and hydrolysis occur. Then we have

$$P/u = f(A/u),$$

where

$$f'(A/u) > 0,$$

and

$$f''(A/u) < 0.$$

From this it can be shown that

- (a) for constant A , P is an increasing function of u , provided $f'(0)$ is finite;
- (b) for constant u , P is an increasing function of A ; and
- (c) for constant u , $\partial^2 P / \partial A^2 < 0$.

Hence, if $f(0) = 0$, for a series of values of u we have a family of P - A curves,

- (a) passing through the origin;
- (b) concave to the A -axis; and
- (c) ascending more steeply the higher the value of u .

If $f(0) > 0$, the curves would have the properties (b) and (c), but they would not pass through the origin: when $A = 0$, they would cut off intercepts on the P -axis increasing with u ; and the convergence of the P - A curves as A approaches zero, for a given pair of values of u , would be less than when $f(0) = 0$. This state of affairs would arise if there were a constant concentration of protein that did not enter into the relationship.

It would be difficult to determine whether property (a) applies to the system we are studying. But there was evidence in paper I that property (b) holds, if we assume that, when U is constant, u also remains constant, since a significant negative A_R^2 term was found in equation (1), Table VII (paper I),

¹ Except in a few aberrant cases, specified in paper II, the dry weight of the plants in our experiments was not significantly affected by treatment and did not drift significantly with time during the period of observation in the cabinets.

and a significant negative A_T^2 term in equation (10). Much stronger evidence for property (β) is found in experiment IV described in the present paper, since the concavity of the P - A_T curve at constant U can be detected visually in Fig. 2, and is also expressed in equation (2), Table II. It is difficult to tell whether property (γ) holds: we have fitted the data of experiments I to III to regressions of the form

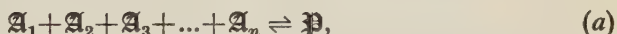
$$P = a + (b_1 + b_2 A)U,$$

but the values of V are little different from those of the equations already given; the fact that U is probably only an anamorphic measure of u adds to the difficulty of demonstrating this property.

It may be concluded that there is accumulating evidence that the $[P]$ - $[A]$ curve is concave to the $[A]$ axis, and this in itself must cause the P - A relation to be affected by U in the direction observed, assuming U to be a measure of u . A specific effect of water could of course be superimposed, i.e. there could be a different concave $[P]$ - $[A]$ curve for every value of U .

On changing proportions of different amino-acids.

The formation of proteins from amino-acids may be conventionally expressed by the equation

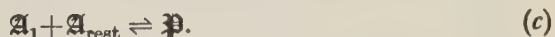


where $\mathfrak{A}_1$, &c., are amounts of amino-acids, and $\mathfrak{P}$ is amount of protein, all expressed in molecular proportions. It follows that, at equilibrium,

$$[\mathfrak{P}] = K[\mathfrak{A}_1][\mathfrak{A}_2][\mathfrak{A}_3] \dots [\mathfrak{A}_n], \quad (b)$$

the square brackets signifying concentrations. Now $[P]$, being measured in terms of total protein-nitrogen, is proportional to $[\mathfrak{P}]$, provided only one molecular species is concerned; and $[A_T]$, being measured in terms of α -amino-nitrogen, of which generally only one atom occurs in each amino-acid molecule, must be approximately proportional to $[\sum \mathfrak{A}]$: if, therefore, equation (b) holds, the curve obtained by plotting $[P]$ against $[A_T]$ can readily be seen to be convex to the $[A_T]$ axis.

This relation holds only so long as the ratio of the concentration of each amino-acid to that of the others remains constant. Equation (a) may be rewritten



If for any reason the concentration of one or more amino-acids, e.g. $[A_1]$, decreases, this leads to an increase in $[A_{\text{rest}}]$ and a decrease in $[P]$: the form of the $[P]$ - $[A_T]$ relation at equilibrium thus depends on the change in the amounts of the various amino-acid components, and under certain conditions the curve could become concave to the $[A_T]$ axis.

It is possible that, with decrease of water content of the leaves, the rate of formation or of disappearance of one or more amino-acids is so affected that its concentration decreases, while the concentration of other amino-acids increases; this could result in the $[P]$ - $[A_T]$ curve becoming concave to the $[A_T]$ axis.

Whatever the form of the steady state $[P]$ - $[A_T]$ relation at constant U , that is, whether it is the relation of equilibrium concentrations, or whether $[P]$ is maintained above the equilibrium concentration by expenditure of energy, such an effect of U on the amino-acids could account for U being a parameter in the relation. However if, when $[A_T]$ increases at constant U , the concentration of one particular amino-acid decreases, then the $[P]$ - $[A_T]$ curve could be concave to the $[A_T]$ axis, and there may be only one such curve, independent of the value of U .

The data of experiment III suggest that there is in fact considerable change in the ratio of the amounts of one amino-acid, viz. cystine, to that of the others as the water content decreases. It is clear from Fig. 3 that the amount

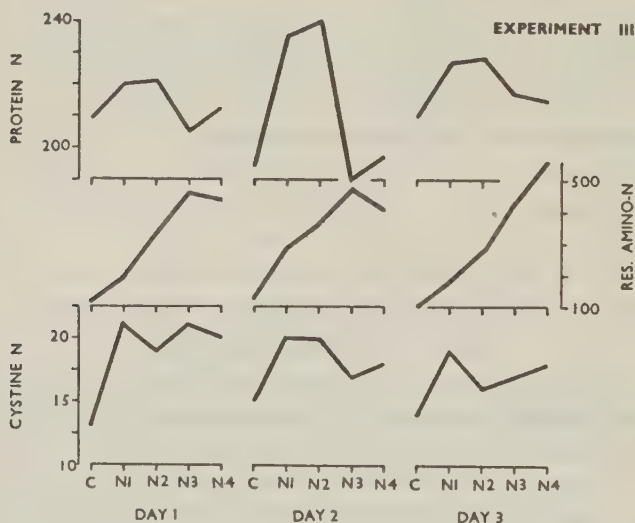


FIG. 3. Treatment effects on the contents of various components of the leaves, experiment III. The value of cystine-N for N_3 , day 3, is missing. All quantities are expressed in mgm. N per 100 gm. dry matter. (The values for P and A_R are taken from Table VI, paper I, and the cystine data from unpublished work of J. G. Wood and B. S. Barrien.)

of cystine does not continuously increase, but may actually decrease, as the amount of the other amino-acids increases. The cessation of increase in the amounts of cystine present are associated with decrease in U , so that we cannot determine whether the amount of cystine would behave similarly with increase in that of the other amino-acids when U is constant. Possibly when U decreases cystine is oxidized at a rate greater than that of its production from sulphate and ammonium ions.

This change in proportion of cystine and other amino-acids must affect the $[P]$ - $[A]$ relation, although to what extent cannot be determined at present: these data merely indicate that at least one amino-acid does not always increase with increase in others.

SUMMARY

There are two ways in which water content may produce an effect on the relation between the amounts, expressed on a dry-weight basis, of proteins and amino-acids: the effect may be the result of changes in the concentrations of these compounds; or change in water content may specifically alter the rate of one or more reactions in the system.

The concentration effect is further discussed: it could arise if the curve relating the concentrations of proteins and amino-acids in the cells were concave to the amino-acid-concentration axis. Evidence that this is the case is brought forward, partly from a further experiment that is here placed on record.

It is shown that, not only does the water content of the tissue affect protein synthesis and hydrolysis, but also the amount of protein is probably a factor determining the water content.

Decreasing water content could also lead to protein hydrolysis if it caused a decrease in concentration of one or more amino-acids. In this way water might have a specific effect. If, however, the decrease in concentration of one amino-acid was associated only with increase in concentration of the total amino-acids, then the effect of water might be purely a concentration one. The difference between the two types of effect would be expressed in the presence or absence of water content as a parameter in the relation between the concentrations of amino-acids and proteins.

It is shown that the amount of one amino-acid, viz. cystine, does not always increase, and sometimes actually decreases, when the amount of the other amino-acids increases; however, in the experiment in which this was demonstrated, water and amino-acid contents were negatively correlated, so that it cannot be concluded whether decreasing water content directly, or increase in concentration of the amino-acids, is associated with cessation of increase in cystine content.

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Studies in the Proteaceae

I. Cytology and Floral Morphology of *Grevillea robusta* Cunn.

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With twenty-six Figures in the Text

INTRODUCTION

IN the literature on the Proteaceae there is a little information on details of floral morphology and the development of the seed. The outstanding recent contributions, including those reviewed by Schürhoff (1926) and Schnarf (1929, 1931), are by Ballantine (1909) on *Protea Lepidocarpon*, Messeri (1928) on *Grevillea macrostachya*, and Brough (1933) on *G. robusta*. The results of these are to a large extent conformatory, but a number of highly interesting structural variations seem to have been overlooked or neglected. The investigation by Brough (1933), however, is very exhaustive, dealing in detail with the development of the gametophytes and the embryo and including a valuable discussion on the systematic position of the family.

The Proteaceae comprise fifty genera and about a thousand species, 'which have a characteristic distribution; there are in Australia 591, East Asia 25, New Caledonia 27, New Zealand 2, Chili 7, tropical South America 36, south-west Cape Colony 262, Madagascar 2, mountains of tropical Africa 5 (Engler). The great majority live in regions where there is annually a long dry season. Correlated with this is the fact that they are mostly xerophytes. . . . Many have their pollen freely exposed, though they are not wind-fertilized (*wind-pollinated*)—a peculiarity perhaps connected with their life in a dry climate' (Willis, 1931). But Sargent (1930, cited by Brough, 1933) states that the family, as a whole, 'has a strong preference for the moister regions' of the island continent of Australia, which forms a conspicuous centre of the Proteaceae, as is evident from the distribution of the species.

Grevillea is the largest genus with about 160 species; the one under discussion here, *Grevillea robusta* Cunn., the Silky Oak, is planted freely, on account of its ornamental value, in many private grounds, as well as in public recreation parks in and about Bangalore. The trees are in full bloom, with conspicuous orange-coloured flowers forming dense inflorescences, during the months from November to April, when plenty of material is readily available for study.



FIGS. 1-14. Fig. 1. Portion of wall of a nearly mature anther, showing the disappearing epidermis, endothecium, three wall-layers, and the large vacuolate tapetal cells. $\times 630$. Fig. 2. Pachytene stage in the nucleus of the microspore mother-cell. Note the ten unequal pairs of threads showing definite orientation. $\times 2,700$. Fig. 3. Diplotene showing the quadrivalent nature and the chiasmata. $\times 2,700$. Fig. 4. Early diakinesis. In some pairs the chiasmata have yet to be terminalized. $\times 2,700$. Fig. 5. Early metaphase. $\times 2,700$. Figs. 6-8. Early and late anaphase. $\times 2,700$. The chromosome pairs have been laterally separated while drawing in Fig. 7. Figs. 9 and 10. The full haploid set in metaphase and anaphase stages

In the course of the following investigation of *Grevillea robusta* Cunn., a preliminary note of which has already appeared, it was originally the intention of the author to describe only certain aspects of floral morphology not previously studied by Brough (1933), but later the scope of the paper was widened to include also some very essential departures from his observations. The material was collected at different stages of development and fixed in Bouin's fluid, which was quite satisfactory, yielding uniformly good results. The usual processes of dehydration and infiltration were followed and sections were cut at varying thicknesses from 10μ to 16μ , according as they were required at different stages of development of the floral parts. An aqueous solution (0.5%) of Heidenhain's iron-alum haematoxylin was employed in staining the sections, by which sufficient contrast and clarity were obtained.

ANTHER AND MEIOSIS IN THE MICROSPORE MOTHER-CELLS

The anther contains four sporangia, derived from four groups of archesporial cells. The latter, after giving rise to the primary parietal layer, form a single-layered plate-like sporogenous tissue. The primary parietal layer divides and gives rise to the endothecium, three middle layers of the wall of the anther, and lastly the tapetum, as described by Brough (1933), whose figures however, show only two middle layers. The tapetal cells are very large and generally more conspicuous than the sporogenous cells themselves. Each tapetal cell has two or more nuclei and a dense mass of cytoplasm, containing one or two large vacuoles. The cells are very active during the meiotic stages of the microspore mother-cells, this activity declining only with the formation of the pollen grains. During the period of the activity of the tapetal cells the cells of the middle layers are usually crushed and become tangentially elongated. During advanced stages of the anther the epidermis becomes largely obliterated and is finally lost, so that the endothecium becomes, for all purposes, the outermost layer of the mature anther (Fig. 1). This feature is perhaps associated, as suggested by Brough (1933), with the hot, dry climate prevailing at the time of anthesis of the flowers.

The single-layered sporogenous cells function directly as the microspore mother-cells, which are at first distinctly polygonal in outline, but become spherical with the initiation of the meiotic changes in their nuclei. With the beginning of meiosis, the chromatin of the nucleus forms the pachytene threads, which are distinctly paired and show large chromatin aggregations. Ten pairs of pachytene threads were invariably counted in a number of nuclei that were examined. The paired threads are of unequal length, and seem to be oriented inside the nuclear membrane more or less definitely with reference to the nucleolus, towards which they tend to be directed (Fig. 2). The

respectively. $\times 2,700$. Fig. 11. Interkinesis after first division. $\times 1,800$. Figs. 12 and 13. Young ovules showing the megaspore mother-cell and the origin and development of the integuments. $\times 450$. Fig. 14. Early eight-nucleate embryo-sac with part of surrounding nucellus. $\times 900$.

pachytene threads next enter the diplotene stage, when the quadrivalent nature of the pairs becomes very evident and the chiasmata are established. As at the preceding stage, exactly ten pairs were constantly seen. These are again of unequal lengths, four being a little longer than the rest (Fig. 3). The number and distribution of the chiasmata are different in the long and short pairs. Each short pair has a single median chiasma, while in the longer pairs there are two or three which are median and terminal, or subterminal, or both. During the succeeding stages there is a reduction in the number of the chiasmata in the longer pairs, and a shifting of their positions towards the ends, in both long as well as short pairs, so as to form the diakinetid chromosomes. These are very highly condensed and intensely homogeneous structures, but showing nevertheless a clear paired nature. These pairs of chromosomes are usually equidistant from one another (Fig. 4). This condition is presumably due, as suggested by Gates (1909) long ago, and recently by Lawrence (1931, cited by Raghavan, 1938), to a mutual repulsion between the pairs, which exists even in the earlier prophase stages. But, with the disappearance of the nuclear membrane the pairs become crowded in the centre and a large number of spindle-fibres make their appearance all round (Fig. 5). The fibres shift their positions and gradually orient themselves so as to form a typically bipolar spindle, with the chromosome pairs occupying the equator.

The bipolar spindle as seen in profile is very narrow, but the fibres are quite conspicuous (Fig. 6). The separating chromosomes show slight size differences, with the spindle-fibres attached towards one end (Figs. 6 and 7). The chiasmata seem to undergo terminalization fairly regularly by the time the chromosomes arrive on the equator of the spindle, as no evidence of lagging could be detected in any of the pairs at the anaphase separation (Figs. 6 and 8). During early metaphase, metaphase and anaphase stages of the first division the chromosome number was verified and found to be ten (Figs. 5, 9, and 10), corresponding to the number of paired threads in the earlier stages, and confirming the haploid number of chromosomes determined by Brough (1933).

After the first division is over there intervenes a brief period of interkinesis (Fig. 11). The succeeding stages in the formation of the tetrad nuclei are quite normal.

OVULE AND DEVELOPMENT OF THE EMBRYO-SAC

The ovary consists of a single carpel, the inrolled margins of which bear two anatropous ovules. Each ovule takes its origin as a nucellar primordium, in which are soon evident a single archesporial cell and the formation of the two integuments. The archesporial cell gives rise to the megaspore mother-cell, and a primary parietal cell which immediately divides by a periclinal wall to form two wall-cells (Fig. 12). By the time the prophase stages begin in the nucleus of the megaspore mother-cell the two integuments, which grow in the meanwhile fairly rapidly, become very apparent (Fig. 13). On the other

hand, Brough (1933) remarks that a noteworthy feature of this ovule is the relatively late inception and slow development of the integuments; this is not borne out by the present investigation.

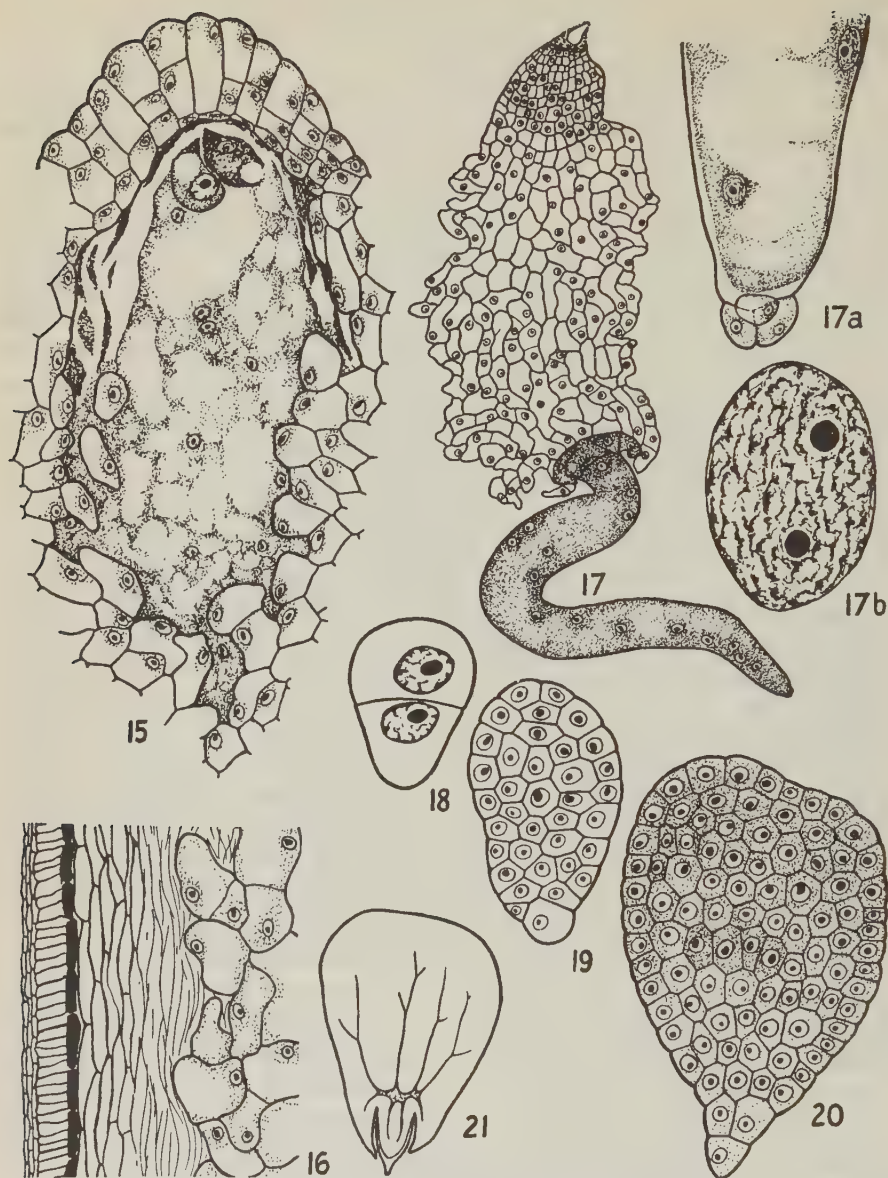
The subsequent stages in the development of the megaspore mother-cell and the formation of the embryo-sac proceed along normal lines (Fig. 14), as stated by Brough (1933), who has followed the entire sequence of events in sufficient detail. The mature ovule has a large mass of nucellar tissue completely invested by the two integuments, the inner of which forms a large micropyle in the form of an inverted funnel. The apex of the nucellus fits into this micropyle as a beak-like projection made up of papillate, glandular cells (Figs. 15, 22, 23, and 24). The inner epidermal cells of the integument bordering on the micropyle, as also those extending down to about one-third the length of the integument (Fig. 22), but in the maturing seed to the base (Fig. 23), have dense contents, presumably tannin. Such tannin-filled cells are also found at the chalazal region of the ovule, where in advanced stages they form a conspicuously dark-brown mass (Figs. 23 and 25).

The papillate cells of the apex of the nucellus are stated by Brough (1933) to be eminently suited to guide the pollen tubes towards the embryo-sac. It may, however, be stated here that in addition to this these cells play an important part in the nutrition of the micropylar end of the embryo-sac prior to fertilization. After fertilization the embryo-sac grows enormously in length and reaches the large mass of nutritive cells situated in the chalaza (Figs. 15 and 23). The papillate cells of the nucellus, though to some extent depleted of their contents in later stages, are quite intact even in the developing seed (Fig. 24). Brough (1933), however, remarks that these are crushed at the time of the passage of the pollen tubes into the embryo-sac and most of his figures of subsequent stages show the nucellus absent and a protrusion of the endosperm cells into the micropyle (cf. his figs. 73, 78, and 80 to 82). In present investigation, however, the apex of the nucellus is always clearly seen in proper median sections of the micropylar region of the older ovule.

The chalazal region of the ovule has meristematic cells which begin to divide actively as the development of the ovule proceeds and the seed begins to be formed (Fig. 25); consequently, a large pad-like tissue of cells arises. These cells are nutritive in function and are used up by the embryo-sac during its post-fertilization stages of growth. The destruction of this tissue, as also of the other nucellar cells at the sides of the embryo-sac, is so complete that in the growing seed a large cavity is formed inside the inner integument, with the nucellus forming only a thin lining layer. Within this cavity the endosperm, enclosing the developing embryo, is freely suspended (Fig. 23) and may be lifted out with a little care.

ENDOSPERM

After fertilization a noteworthy feature is the behaviour of the embryo-sac, which grows enormously in size by encroaching on and dissolving the



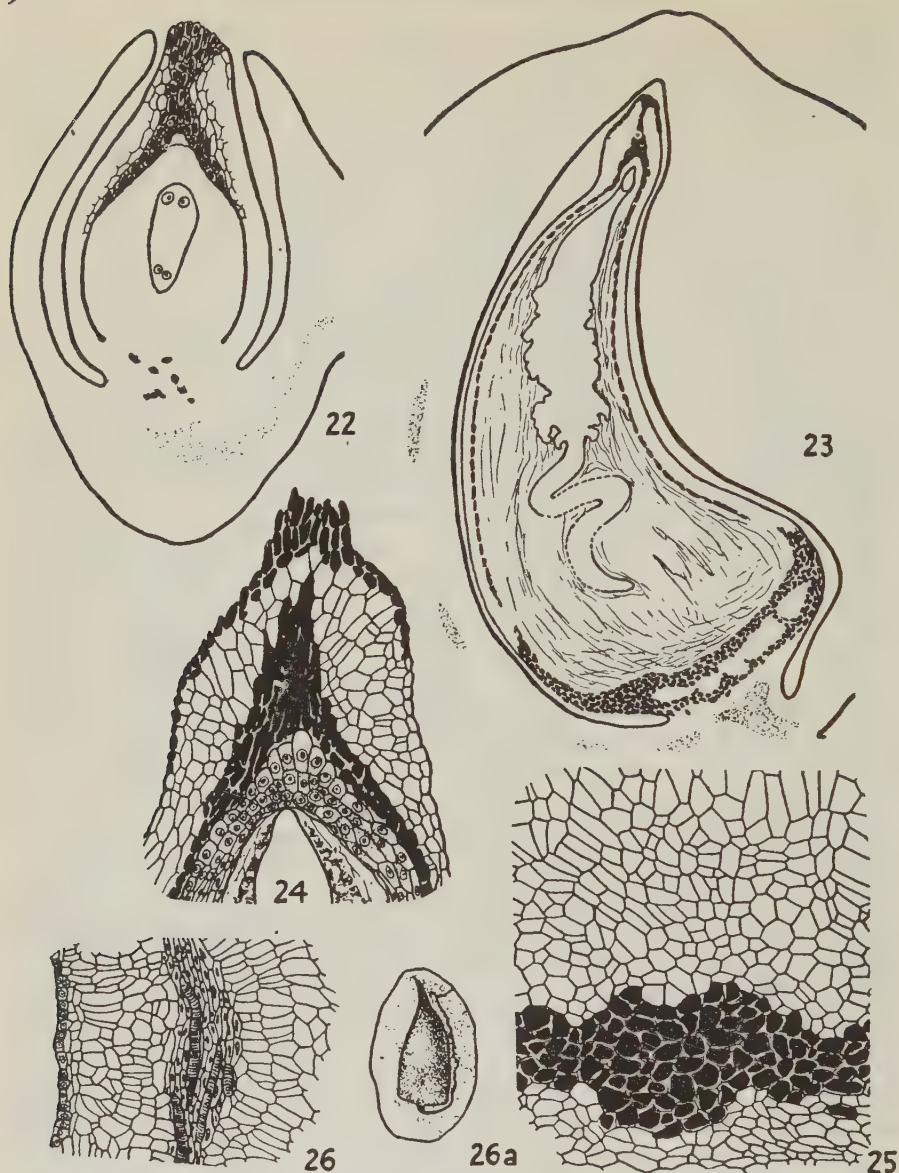
FIGS. 15-21. Fig. 15. Embryo-sac after fertilization showing one persisting synergid, the fertilized egg, and a number of free endosperm nuclei. Note the papillate glandular cells of the apex of the nucellus above the micropylar end of the sac and the destruction of the cells of the nucellus all round by the growing embryo-sac, which becomes irregular in contour. $\times 450$. Fig. 16. The peripheral cells of the endosperm of the central region of the embryo-sac forming invading processes and destroying the nucellar cells. Part of the inner integument showing its entire thickness and the layer of tannin cells is indicated in the figure. $\times 200$. Fig. 17. A whole mount of endosperm separated from a developing seed to show the cellular

surrounding nucellar cells. It becomes irregular in outline, and this is accompanied by the formation of free endosperm nuclei (Fig. 15). These nuclei steadily increase in number with continued growth of the embryo-sac and finally organize themselves to form the endosperm tissue. But the process of cell formation does not proceed uniformly throughout the embryo-sac, in which three distinct regions—upper, central, and lower—may easily be recognized. The formation of endosperm tissue is restricted to the upper and central portions of the sac, which show small, regular, compactly arranged cells and larger, irregular, and loosely arranged cells, respectively. The lower region of the embryo-sac fails to form a tissue, but includes merely a number of free endosperm nuclei. This is in conformity with the statement of Brough (1933) that endosperm formation is confined to the upper two-thirds of the embryo-sac, while it proceeds slowly towards the chalazal region, which is seen to be devoid of tissue and so becomes collapsed in mature seeds. Before the lower region of the sac suffers this collapse it displays a striking phenomenon, which has been altogether overlooked by Brough.

While the endosperm cells of the upper region of the embryo-sac meet the immediate nutritive demands of the young developing embryo, the peripheral cells of the central portion, consisting of large and loosely arranged endosperm cells, grow out as lateral processes which invade the surrounding nucellus (Fig. 16) and gradually destroy the cells so completely that a large cavity is finally formed in the developing seed. A thin peripheral layer of nucellus may, however, persist to some extent as an internal lining of the integument. The invading cells of the endosperm are remarkably similar to the peripheral cells of the foot in the sporophyte of *Anthoceros*, a comparison with which becomes more striking on a consideration of the similarity of their roles in actively absorbing materials from the surrounding cells.

The lower region of the embryo-sac, in which the formation of endosperm tissue is totally suppressed, reveals a unique feature in its subsequent behaviour. It assumes a pronounced haustorial function and develops into a peculiar tubular structure, which is very long, often measuring as much as 3 mm., and is coiled. This structure is strikingly vermiform in appearance (Figs. 17 and 23) and so may be appropriately designated the *vermiform appendage* of the endosperm (Kausik, 1938). The appendage has a very dense mass of highly vacuolate cytoplasm containing a number (40–50) of large nuclei, and showing at its proximal region curious horn-like processes, which are invariably four in number. Distally the *appendage* is more or less pointed

regions and the coenocytic *vermiform appendage*. $\times 35$. Fig. 17a. The tip of the *appendage* showing the three persisting antipodal cells. $\times 450$. Fig. 17b. One of the free nuclei of the *appendage* showing the high chromatin content. $\times 900$. Figs. 18–20. Some stages in the development of the embryo. Note the clear distinction into the tapering proximal and broad distal regions. 18, $\times 1,350$; 19 and 20, $\times 450$. Fig. 21. A fully developed embryo from a mature seed showing one of the cotyledons with its basal lobes, the hypocotyl, and the radicle with the root-cap.



FIGS. 22-6. Fig. 22. Outline of the longitudinal section of a young ovule containing a four-nucleate embryo-sac. $\times 160$. Fig. 23. Longitudinal section of a seed showing in outline the position of the endosperm and its appendage. $\times 25$. Fig. 24. The micropyle of above showing the persisting glandular cells of the apex of the nucellus. $\times 80$. Fig. 25. The chalazal region of the seed to show the regularly arranged meristematic cells of the chalaza. $\times 240$. The cells shown black in Figs. 22-5 contain tannin. Note the difference in the distribution of these cells in early and late stages as shown in Figs. 22 and 23. Fig. 26. Portion of the outer epidermis showing dense contents. $\times 80$. Fig. 26a. An entire seed showing the broad membranous wing and the vascular strand on the side of the funiculus. $\times 1.5$.

and shows three small cells (Fig. 17a); the latter are evidently the persisting antipodals, as may be judged from their disposition.

The *vermiform appendage* increases rapidly in length by digesting the nucellar cells on its way; ultimately it reaches the large mass of nutritive cells situated in the chalaza. These cells are used up completely by the advancing appendage and a large cavity is thus formed in the chalazal region, which shortly before showed extensive and compact tissue (Fig. 23). In the extraordinary haustorial activity of the *appendage* the free endosperm nuclei seem to play a considerable part, for they become very conspicuous and show a high chromatin content (Fig. 17b). They are usually oval in shape and include one or two nucleoli. Each nucleus may measure about 50μ along its larger diameter.

The *vermiform appendage* begins to decline in activity after the destruction of all the nucellar cells and is in a collapsed state during subsequent stages of the formation of the seed. In earlier stages it is possible, as already stated, to separate from the integument the entire mass of endosperm with the *appendage* by a little manipulation of the seed at the proper stage. This mass of endosperm may then be mounted whole and stained for examination, when a complete and clear picture of it is readily available (Fig. 17).

EMBRYO

The development of the embryo, compared with that of the endosperm, is relatively slow in the earlier stages. Later it progresses rapidly at the expense of the endosperm, which in the immediate vicinity shows darkly staining cells in the process of breaking down. The apical region of the endosperm happens therefore to be the earliest to be used up by the young embryo, and so could not be identified as an intact mass of cells projecting into the micro-pyle as figured by Brough (1933).

As development proceeds, the embryo becomes progressively larger, and consists of more cells (Figs. 18–20). The dermatogen, periblem, and plerome are differentiated gradually and the embryo becomes conspicuously wedge-shaped towards its proximal end; distally it is broader and rounded in contour, where the cells have dense contents (Figs. 19 and 20). The shape of the embryo as it develops does not change from an oval to a spherical shape, as assumed by Brough (1933). The fully developed embryo (Fig. 21) consists of two large cotyledons (each of which is provided with two basal lobes at the point of its insertion on the hypocotyl), a stem tip and a massive radicle having a pointed root-cap.

DEVELOPMENT OF THE SEED

In comparing the conditions met with in *Grevillea robusta* with those in *Protea Lepidocarpon* R. Br. (Ballantine), Brough (1933) mentions that the meristematic cells at the chalazal region of the ovule are active until the time of fertilization, but elsewhere in the paper states that a striking feature of the

seed, consequent on fertilization, is a rapid increase in its dimensions, particularly in a plane parallel to the longitudinal axis of the seed, this being due for the most part to the great activity of the meristematic cells at the chalaza. With continued development, the two seeds of the ovary become much flattened in an antero-posterior plane, accompanied by the formation of a wing, which is thin and papery.

In the developing seed the inner integument, with its tannin-containing inner epidermal cells, takes a large part in forming a fairly hard and protective covering for the growing embryo inside (Fig. 23). The other cells of the integument become crushed and constitute an internal lining for the wing, which is solely formed by the outer integument in which the cells divide a number of times tangentially and form several layers of radially elongated cells (Fig. 26). The cells of the inner epidermis of the outer integument become more or less glandular. It is difficult to offer any suggestion as to the role of these cells.

The funiculus of the ovule is also incorporated in the wing, which therefore consists, on one side, of the fused outer integument and the funiculus, where the conspicuous vascular strand of the latter is seen (Fig. 26 and 26a).

CONCLUSIONS

A detailed study of the meiotic divisions of the nuclei in the microspore mother-cells in *Grevillea robusta* has revealed not only the paired condition of the chromatin threads during the prophase stages but also the quadrivalent nature of these pairs at certain stages. Despite the smallness of the nuclei the material has lent itself fairly satisfactorily, doubtless on account of a small haploid number of chromosomes, to an examination of the meiotic cycle. To summarize, this cycle shows a definite number of paired threads in the prophase nuclei, the orientation of the pachytene pairs with reference to the nucleolus, the unequal lengths of the paired threads at the prophase stages, and a corresponding size difference, though slight, in the chromosomes at the later stages of the first division, the number and nature of the chiasmata, and lastly, the separation of the anaphase chromosomes.

Regarding the morphology of the flower in general a discussion has already been entered into by Brough (1933), who also includes an admirable survey of pollination and a review of the taxonomic position of the family. He concludes that the Proteaceae may be closely related to the Geissolomataceae, and through them to the Thymeleaceae in general. He adds that the simplicity of the floral organs in the Proteaceae may well be primitive, in which case an affinity with some order, lower in the scale of evolution than the Thymeleales, will have to be sought. A discussion of the systematic position of the family is not here undertaken but, on the other hand, certain features of floral morphology, which do not agree with the descriptions of Brough (1933) or are not described by him, are given here. The more important of these are the origin of the integuments, which occurs fairly early, the persistence of the glandular cells at the apex of the nucellus till a very late stage in the development of the

seed, the early destruction of the apex of the endosperm, which does not project into the micropyle, and the wedge-shaped embryo, which shows no change in shape from an oval to a spherical form, as assumed by Brough (1933). An interesting feature, which was not described by Brough (1933), is the peculiar and unique organization of the endosperm.

Attention may here be drawn to the fact that the utilization of nutritive material in the neighbouring cells, and available for the growing embryo-sac particularly after fertilization, which is so complete and thorough in *Grevillea robusta*, recalls to some extent the condition met with in *Utricularia* (Wylie and Yocom, 1923; Kausik, 1935, 1938). In the latter case, the role of absorption is assigned to definite portions of the embryo-sac, namely the haustoria, which invade the placental and chalazal nutritive tissues. In *Grevillea robusta*, on the other hand, the embryo-sac, as such, is very active and destroys the surrounding nucellar cells after fertilization. Later, with the differentiation in the endosperm into the cellular region and the lower *vermiform appendage*, contemporaneous with which is also the formation of the marginal cells of the endosperm as absorptive processes, fresh invasions are made of the chalazal cells and the remaining peripheral layer of nucellus. The result of this extraordinary post-fertilization activity of the embryo-sac, when it may be said to have entered the second phase of its growth, is a wholesale destruction of all the cells of the nucellus. This leads to the formation of an immense cavity inside the seed, within which is the endosperm suspended freely from the micropyle.

In concluding this investigation, it may be stated that *Grevillea robusta* reveals in its morphology a number of distinct and unique features. The most striking of these is undoubtedly the peculiar formation of the endosperm. It would be highly interesting to determine if similar features are also present in the other members of the Proteaceae. A detailed examination, similar to that here completed, of as many genera as possible would thereby form a valuable contribution to our knowledge of the family, in view of the fact that the members constituting it are so diverse and are distributed in widely separated regions of the world. Such a study should also throw light upon the position of the family which is at present rather uncertain. It is the intention of the author to study as many members of the Proteaceae as possible, when it is also hoped to include a discussion of the important facts of floral morphology.

SUMMARY

A detailed account of the cytology and floral morphology of *Grevillea robusta* Cunn. is given.

The meiosis in the nuclei of the microspore mother-cells has been followed in detail and the haploid number of chromosomes reported by Brough (1933) has been confirmed.

The formation of a peculiar coenocytic tubular structure at the lower end

of the endosperm and designated the *vermiform appendage* is described at length. In addition, attention is also drawn in the paper to the marginal absorbing cells of the endosperm as processes invading the nucellus. These structural features of the endosperm have not been previously reported, either in this or any other plant.

Several aspects of the life-history have been discussed with special reference to the earlier work of Brough (1933) on *Grevillea robusta*.

The author takes this opportunity to record his sincere thanks to Dr. M. A. Sampathkumaran, Professor of Botany, University of Mysore, for advice and kind encouragement during the course of this investigation.

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The Interaction of Factors in the Growth of Lemna

XIII. The Interaction of Potassium and Light Intensity in Relation to Root Length

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With two Figures in the Text

INTRODUCTION

THE effect of variation in nitrogen supply at different light intensities on the root growth of *Lemna* colonies has been considered in a previous communication (White 1937a), in which the conclusion was drawn that variation in length of root is a sensitive indication of variation in carbohydrate-nitrogen balance within the frond. A high ratio of protein to starch was associated with a decrease of root length relative to frond dry weight whereas a high ratio of starch to protein was associated with an increase of root length relative to frond dry weight. It was suggested that when nitrate supply is high relative to assimilation rate sugars are mainly used for protein synthesis and in the development of new fronds so that the rate of translocation of sugars to the root meristem is slow, leading to retardation of root growth; when nitrate supply is low relative to assimilation rate a high level of surplus sugar in the frond accelerates the rate of translocation of sugars to the root meristem and root growth is relatively rapid. The present communication records data of the effect of variation in potassium supply on root length at different light intensities.

EXPERIMENTAL PROCEDURE

Colonies of *Lemna minor* were exposed, at a controlled temperature of 25° C., to continuous illumination from gas-filled water-cooled lamps, which were adjusted from the reading of a Holophane lumeter to give four intensities of light—300, 180, 120, and 60 ft.-candles. These colonies, obtained by division of a single clone, floated upon nutrient solution of inorganic salts¹ and glass-distilled water in small beakers (30 c.c.) of Pyrex glass. The colonies were transferred to duplicate beakers containing fresh nutrient solution at twelve-hour intervals for a period of twenty days. The numbers of fronds of

¹ $\text{CaH}_4(\text{PO}_4)_2$ 0.101 gm.; MgSO_4 0.255 gm.; $\text{Ca}(\text{NO}_3)_2 + 4 \text{Aq}$ 0.291 gm.; $\text{Mg}(\text{NO}_3)_2 + 6 \text{Aq}$ 0.191 gm.; Fe_2Cl_6 0.002 gm.; distilled water 1 litre; K_2SO_4 equivalent to potassium supply of 200, 2.0, 0.125, and 0 mg. per litre.

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the colonies were adjusted periodically to the size of the beakers, by the random removal of plants, to prevent overcrowding. Growth of the colonies was studied at four potassium levels of the nutrient solution—200, 2·0, 0·125 mg. potassium as sulphate and no potassium additional to that leached from the glass of the beakers. An analysis of the growth data obtained and further details of the technique used is being presented in a separate communication of this series (in the press).

EXPERIMENTAL RESULTS

Length of root, after twenty days' growth under all combinations of four light intensities and four potassium levels of the nutrient solution, is shown in Table I with standard errors of the means of samples of eight plants of approximately equivalent age.¹ Decrease in root length is clearly associated with reduction in either light intensity or potassium supply.

TABLE I

Length of Root (cm. with S.E. of mean) of Colonies Subject to All Combinations of Four Light Intensities and Four Potassium Concentrations

Light (ft.-candles).	Potassium (mg. per litre).			
	0·0	0·125	2·0	200
300	0·18±0·03	0·41±0·06	1·23±0·05	1·45±0·04
180	0·20±0·01	0·23±0·02	0·56±0·03	0·63±0·04
120	0·17±0·01	0·21±0·01	0·38±0·02	0·45±0·03
60	0·08±0·01	0·11±0·01	0·10±0·01	0·13±0·01

The relation between length of root and potassium supply at four levels of light intensity is shown graphically in Fig. 1. Continuous curves represent measurements (Table I) made at the end of the experiment (day 20) and broken curves represent measurements made half-way through the experiment (days 8, 9, and 10). Fig. 1 shows that the interaction effect of the factors is not constant. As the experiment proceeds root length at the two lower potassium levels is progressively decreasing, but at the higher potassium level is progressively increasing. The detrimental effect of low potassium supply on root length at the maximal light intensity of 300 ft.-candles is not detected at the minimal light intensity of 60 ft.-candles.

The relation between length of root and light intensity for four levels of potassium supply is shown graphically in Fig. 2. All curves (save possibly that for the colony with lowest potassium level) are linear, the slopes being determined by the different potassium levels. The shifting of the interaction effect as the experiment proceeds may be estimated from comparison of the regression coefficients at the half-way stage of the experiment with those at the end of the experiment. These are given in Table II. It is evident that low potassium level has a progressively detrimental effect on root length whereas high potassium level has a progressively beneficial effect.

¹ In order to straighten the roots for accurate measurement the plants were transferred to damp blotting-paper with a camel's hair brush.

In a previous communication (White, 1937*b*) the ratio between dry weight of plant and length of root was taken as a measure of relative root development. Dry weight of the plants that provided the measurements given in Table I was obtained by drying *in vacuo* under standard conditions, as

TABLE II

Regression Coefficients of Root Length on Light Intensity of Colonies with Four Potassium Levels. The Values Represent Increase (cm.) for a Rise of 100 ft.-candles

Potassium (mg. per litre).	After 8–10 days treatment.	After 20 days treatment.
200	—	0·54
2·0	0·40	0·47
0·125	0·32	0·12
0·0	0·27	0·04

described by Su and Ashby (1929), and weighing on a microchemical balance. The roots were also dried and included in the weighing, but as their contribution to total weight is known to be only of the order of 14 per cent.¹ the values obtained cannot have been materially influenced by differences in root development and represent virtually the dry weight of the fronds. The effect on relative root development of decreasing potassium supply at the maximal experimental light intensity of 300 ft.-candles is shown in Table III. Length

TABLE III

Effect of Decreasing Potassium Supply on Root Length and Dry Weight at a Light Intensity of 300 ft.-candles

Potassium (mg. per litre).	Dry weight (mg.) per plant of two fronds.	Root length (cm.).	Ratio (× 100).
200	0·090	1·45	6·2
2·0	0·086	1·23	7·0
0·125	0·068	0·41	16·6
0·0	0·072	0·18	40·0

of root with 200 mg. potassium per litre is eight times that with no added potassium, whereas dry weight differences are only of the order of 20 per cent. The detrimental effect of low potassium level is thus much more marked on root length than on dry weight.

DISCUSSION

There is a direct relationship between root length and light intensity (Fig. 2). This is conformable with the view that root growth is related to carbohydrate supply, for the curves relating net assimilation rate and light intensity have also been shown to be approximately linear over this range of light intensity

¹ I am indebted to Mr. T. A. Oxley for this determination.

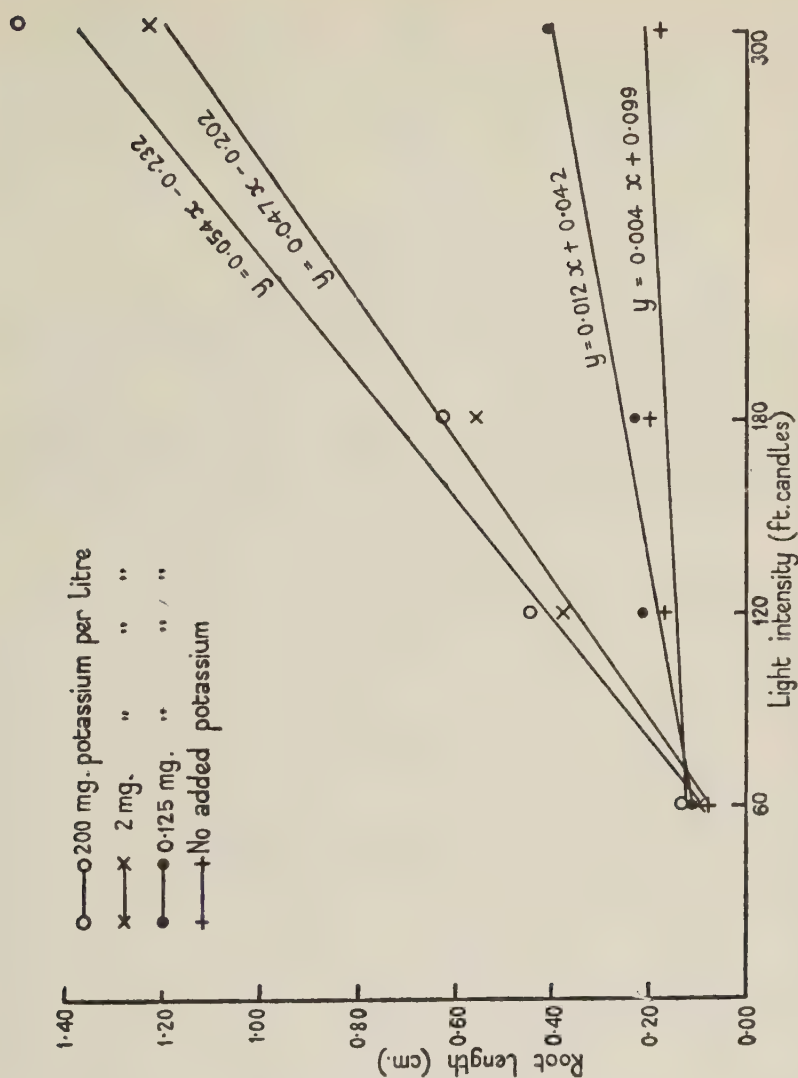


FIG. 2. Length of root plotted against light intensity for four levels of potassium supply. Straight lines have been fitted by the method of least squares.

(White 1937, Fig. 6) in contrast to the more complex relationships found for rate of increase in frond number and area (White 1937, Figs. 2 and 4).

The detrimental effect of decreasing potassium supply on root length is more marked than the corresponding effect on frond dry weight (Table III). This suggests strongly that the high dry weight per unit area and high starch content of the potassium-starved frond (White 1936, Table V and p. 184) are associated with low sugar content, for a relatively high sugar concentration should have increased the rate of supply of sugar to the root meristem and led to a length of root less disproportionate with the dry weight of the frond (White, 1937*b*). The effect of decreasing potassium supply on root growth is compatible with the view (White, 1936) that the starch-sugar balance of the frond is controlled by the potassium level so that with decreasing potassium supply a higher proportion of the carbohydrates formed are present as starch and a lower proportion as sugar.

As the experiment proceeds and the effects of treatment become more pronounced there is an increase in root length of the colonies with high potassium supply and decrease in root length of the colonies with low potassium supply (Fig. 1 and Table II). This decrease of root length with potassium starvation is more severe at high light intensity, the magnitude of the fall during the second half of the experiment (Fig. 1) ranging from over 75 per cent. under 300 ft.-candles to less than 10 per cent. under 120 ft.-candles. This suggests that, if the experiment had been continued for a longer period, the length of root of the potassium-starved colonies would have become less at high light intensity than at low light intensity. Turning to Fig. 2 it is of interest to note that the curves of the colonies with relatively high potassium supply, if extrapolated, would cut the abscissa axis at light intensities of between 30 and 40 ft.-candles, whereas the corresponding curves of the colonies with low potassium supply would cut the ordinate axis. This suggests that, if a light intensity lower than 60 ft.-candles had been used, root length would have been less with high potassium supply than with low potassium supply. Reduction of either light intensity or potassium supply alone clearly leads to *proportionately greater decrease* in length of root than reduction of both factors together, and increase of either light intensity or potassium supply alone to *proportionately less increase* in length of root than increase of both factors together. The basis of these effects, similar to those referred to in a discussion of the interaction of light intensity and nitrogen supply (White, 1937*a*), will be considered in a separate communication in conjunction with estimates of change in frond number, area, and dry weight.

SUMMARY

Variation in root length of Lemna colonies subject to sixteen combinations of light intensity and potassium supply was studied. Length of root (with net assimilation rate) is directly related to light intensity of the order of 50–300 ft.-candles (continuous illumination) at all potassium levels, suggesting that

root growth is closely related to carbohydrate level. Decreasing root length with falling potassium level is associated with falling net assimilation rate but with rising starch content, this suggesting a close relationship between root growth and sugar level of the potassium-starved frond. Reduction of either light intensity or potassium supply leads at all levels to decrease of root length. The magnitude of this fall is relatively less when both factors are decreased, and relatively greater when one factor is decreased and the other maintained at the same level.

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Studies in the Pathogenicity of Tropical Fungi

II. The Occurrence of Latent Infections in Developing Fruits

BY

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I. INTRODUCTION

IN the first paper of this series, Baker and Wardlaw (1937) demonstrated that certain fungi, which cause active rotting of tropical fruits during storage or normal senescence, usually obtain entry at a much earlier stage in the development of such fruit, but remain inactive or dormant until final maturity begins. Both on scientific and practical grounds it would appear that this aspect of fungal parasitism deserves careful investigation. To this end an attempt has been made to determine at precisely what stage in the development of different tropical fruits these latent infections are established, and what ecological factors govern their distribution.

In this paper a survey has been made of the organisms which may occur as latent infections during the development of citrus, mango, avocado pear, papaw, tomato and cacao fruits.

II. PREVIOUS INVESTIGATIONS

Investigations conducted, both in temperate regions and in the tropics, on the development of rots of ripening fruits have led to the conclusion that certain pathogens gain entry into the unripe fruit some considerable time before the fruit is harvested. Latent infections are formed, and are usually invisible when the fruit is picked, only becoming conspicuous during the later stages of ripening.

Shear and Wood (1933) found that cultures of species of *Colletotrichum* and *Glomerella* could be obtained from the apparently healthy leaves, stems, flowers and fruits of many plants if the tissues were surface sterilized and incubated in a sterile container. Using a similar technique, Dastur (1916) found that *Gloeosporium musarum* gained entry into the fruits of the plantain in India while they were immature and green, and did not develop further until the onset of final maturation. A considerable amount of work has also been carried out on apple fruit rots, especially *Venturia inaequalis*, the cause of apple scab disease, and on certain storage rots of citrus fruits, avocado pear, mangoes and other tropical fruits.

Kidd and Beaumont (1925) working with apples, concluded that storage

rots were brought about by spores which remained on the surface of the fruit or in the lenticels until such a time as they were able to germinate and enter the tissues. Other workers have realized that this explanation does not cover all the facts, and that consideration must be given to the presence of dormant infections established prior to harvesting. From the manner in which scab infections develop in storage, Weismann (1930) postulated that these must be present before the fruits are stored. Stahelin (1931) and Faes and Stahelin (1931) reached a similar conclusion and advanced the suggestion that the mycelium of *Venturia inaequalis* must be present in the epidermis while the fruit is still attached to the tree. A. S. Horne and his colleagues (1930), Fulsom (1933), Bratley (1933, 1937), and Wormald (1934), all working with apples in storage, found the manner in which the lesions of scab develop in storage inexplicable unless it is assumed that they are established before the fruit is harvested. In brief, the present position with regard to apple scab investigations is that while many workers have indicated that latent infections must be present prior to harvesting, conclusive proof, based on isolating the organism from superficially sterilized epidermal tissue, has not yet been obtained. On the other hand, in studies of tropical fruits, and using an appropriate mycological technique, definite proof has been obtained that numerous latent infections are present in apparently healthy fruits (Baker and Wardlaw, 1937).

In citrus fruits the skin is smooth and waxy, lenticels are absent, and the small, scattered stomata are incapable of harbouring fungal spores. Infection of lenticels of the type described by Kidd and Beaumont (1925) is therefore precluded. Hawkins (1921) found *Colletotrichum gloeosporioides*, the cause of anthracnose of many tropical fruits, in the skin of apparently sound grapefruit in storage. Fulton and Bowman (1927), working with *Phomopsis citri* and *Diplodia natalensis*, two of the organisms causing stem-end rot of citrus fruit, concluded that these pathogens must already be present in some part of the button before harvesting, and that they subsequently passed into the tissues of the fruit. Ruehle and Kuntz (1931) isolated *Phomopsis citri* from young leaves, fruits, buttons and dead wood of citrus, and produced evidence indicating that the fungus was within the button when the fruit was harvested; and Kuntz (1932) obtained pure cultures of *Phomopsis citri* from the buttons of immature citrus fruit. Tisdale and West (1934) also succeeded in obtaining pure cultures of *Diplodia natalensis* and *Phomopsis citri* from the buttons and stems of tree-ripe oranges and grapefruit. From commercial experience Winston (1935) has inferred that the above stem-end rot pathogens must be established while fruits are still on the tree, and that the fungi are dormant in the stem or stem parts at the time of harvesting. As a result of mycological investigations on S. African grapefruit and oranges, Bates (1936) has also advanced the theory that *Colletotrichum gloeosporioides* must be present in immature fruits as latent infections. Baker and Wardlaw (1937) found *Phomopsis citri* as a commonly occurring latent infection in fruits and

buttons of grape-fruit, but consider *Botryodiplodia theobromae* to be a wound parasite obtaining entry at the cut stem-end at the time of harvesting the fruit.

Working with various tropical fruits, and using the sterilization and incubation technique, Wardlaw (1931) confirmed the work of Dastur (1916) that *Gloeosporium musarum* is present as latent infections in immature bananas, and Wardlaw and McGuire (1932), using a similar technique, showed that *Piricularia grizea*, the cause of pitting disease of the banana, is present in the tissues of green fruit. Similarly, Wardlaw and Leonard (1936) demonstrated the presence of *Colletotrichum gloeosporioides* in the skin of green mangoes and papaws. W. T. Horne (1934), from results obtained in storage, inferred that latent infections of *Dothiorella ribis* must be established in the avocado pear while the fruit is on the tree, and Horne and Palmer (1935) have shown that this organism enters the young lenticels of immature fruit on the tree, and forms mycelium which remains dormant in the air-spaces until the fruit is becoming senescent in storage.

By making large numbers of isolations from apparently sound fruit of all ages, Baker and Wardlaw (1937) have shown that several fungi are of common occurrence in the tissues of many tropical fruits, and have demonstrated that by the time they are harvested all grape-fruit, mangoes, avocado pears and papaws contain the latent infections of several organisms.

III. ORGANISMS CAUSING LATENT INFECTIONS IN TROPICAL FRUITS

1. *Colletotrichum gloeosporioides* (*Glomerella cingulata*). The conidial stage of this fungus can be readily isolated from citrus fruits, papaws, mangoes and avocado pears. Cultures on potato-sucrose-agar occasionally develop the perithecia, asci and ascospores of *Glomerella cingulata*.

2. *Guignardia* sp. This fungus was identified by the Imperial Mycological Institute (to whom the author is indebted for several of the identifications used in this paper) as a species of *Guignardia* hitherto undescribed. It produces a pycnidial stage (*Phyllostictina* sp.), markedly resembling *Phoma citricarpa*, but since it does not appear to cause the Black Spot Disease of citrus, it must be regarded as a distinct species. It has been frequently isolated from the several fruits under consideration.

3. *Phomopsis citri* was frequently isolated from citrus fruit. A species of *Phomopsis* indistinguishable from it has been obtained from papaws, mangoes and avocado pears. It seems probable that *Phomopsis citri* may be regarded as a pathogen common to a wide range of tropical fruits.

4. *Fusarium expansum* was described in the first paper of this series as a possible latent infection. Further research has shown that its occurrence is irregular. It now seems more likely that it possesses resistant spores sometimes capable of withstanding the surface sterilization technique used, and so appearing in the Petri dishes with latent infection fungi.

5. *Dothiorella ribis* has been found occasionally as a latent infection of

mangoes and avocado pears; Horne and Palmer (1935) have described the method by which this fungus penetrates avocado fruits by way of the stomata and becomes established in the air-spaces as a latent infection.

Certain other slow-growing fungi are present occasionally as latent infections; but these await identification.

IV. EXPERIMENTAL METHODS

The technique for culturing surface washings and latent infections has already been described (Baker and Wardlaw, 1937). Plates for exposure in the field were prepared under aseptic conditions and immediately placed in the sterile container in which they were carried to the locality where they were to be exposed. After exposure they were replaced in the container and kept there until examined three days later, after which they were discarded. These precautions were necessary, and only one examination was possible because of the risk of confusing the abundant contaminations of a tropical laboratory with the fungi under investigation.

V. FUNGI ASSOCIATED WITH GRAPE-FRUIT

1. *The distribution of organisms in the orchards.* Isolation of fungi from fruit washings, undertaken at regular intervals for over a year, has yielded the results summarized in Table I. Only organisms which could be identified are discussed; a minority of isolations was discarded as failure to induce sporulation or the development of fruiting bodies, sclerotia, &c., made identification impossible. From the data submitted in this Table it will be seen that an abundant saprophytic fungal flora is present in grape-fruit orchards, and that the modifying effect of season on the constituent members of this flora is negligible. On some occasions certain fungi have been noticeably abundant, e.g. *Acrothecium lunatum* and *Colletotrichum gloeosporioides*. (Trinidad is in the humid tropics: temperature is fairly constant throughout the year and the dry season as a rule is not excessively severe.) The fungal flora obtained by exposing plates in plantations (Table II) was similar to that found on the surface of fruit.

The spores of three common storage pathogens of grape-fruit, *Botryodiplodia theobromae*, *Phomopsis citri* and *Colletotrichum gloeosporioides*, were found fairly regularly, but others including *Penicillium digitatum*, *Dothiorella ribis* and *Guignardia* sp. (the latter produces abundant latent infections, but apparently never causes a storage rot) were not obtained: failure to isolate certain organisms is referable to the presence of unexplored factors and the limitations of the technique employed. Thus *Penicillium digitatum*, although abundant on dropped fruit, was not isolated.

Table III gives the data for washings of fruit obtained from six estates in different areas under different conditions of soil exposure and rainfall. Of an identified flora of nineteen organisms, eleven have been found on five of

TABLE I
Grape-fruit: Superficial Fungi obtained by Washing

Date.	Estate.	<i>Pestalotzia leprogena.</i>	<i>Penicillium italicum.</i>	<i>Penicillium</i> spp.	<i>Fusarium sambucinum.</i>	<i>Cladosporioides herbarum.</i>	<i>Colletotrichum gloeosporioides.</i>	<i>Botryodiplodia theobromae.</i>	<i>Trichoderma lignorum.</i>	<i>Eidemia</i> sp.	<i>Fusarium expansum?</i>	<i>Rhizopus nigricans.</i>	<i>Mucor</i> spp.	<i>Aspergillus</i> spp.	<i>Clasterosporium maidicum.</i>	<i>Oospora</i> sp.	<i>Nigrospora oryzae.</i>	<i>Phomopsis citri.</i>	<i>Acrothecium lunatum.</i>	<i>Acrothecium</i> spp.	<i>Fusarium lateritium.</i>	<i>Popularia sphaerosperma.</i>	<i>Ascomycete</i> spp.	<i>Basidiomycete</i> spp. (<i>Coprinus</i> , &c.)	<i>Sacharomycete</i> spp.	<i>Pseudoplea trifolii.</i>	<i>Hormodendrum cladosporioides.</i>	<i>Fusarium semitectum.</i>	<i>Aspergillus niger.</i>	<i>Pythium</i> sp.	
7/1/36	A	+	+	+	+	+					+			+	+	+	+				+	+	+								
9/1/36	B	+	+		+	+		+	+	+	+	+									+	+	+								
17/1/36	C	+	+		+	+		+	+	+	+	+		+			+				+	+	+								
21/1/36	D	+	+		+			+	+	+	+	+			+	+		+			+	+	+								
29/1/36	E	+	+		+			+	+	+	+	+			+		+					+	+	+							
5/2/36	F	+	+		+			+	+	+	+	+							+			+	+	+							
11/2/36	B	+	+		+	+		+	+	+	+	+	+		+							+	+	+							
19/2/36	C	+	+		+	+					+	+			+						+										
11/5/36	C	+	+		+	+	+		+		+	+	+		+		+	+	+		+			+					+	+	
18/5/36	D	+	+		+			+		+					+						+										
19/5/36	A		+	+				+			+											+	+	+			+				
4/6/36	B	+			+	+		+	+	+	+		+		+		+	+	+			+	+	+							
8/6/36	D	+						+	+	+	+				+		+	+	+				+	+	+						
19/6/36	A	+			+			+			+	+					+	+					+	+	+						
26/6/36	C	+			+	+					+		+				+	+	+		+			+	+					+	
9/7/36	D	+			+			+	+		+		+					+			+			+	+						
13/7/36	B	+				+					+	+					+	+	+		+			+	+						
27/7/36	A	+			+				+		+		+				+	+	+		+			+	+						
5/8/36	C	+	+		+				+		+	+			+		+	+	+		+			+	+						
19/8/36	D	+	+		+			+	+		+	+			+		+	+	+		+			+	+						
11/9/36	A	+	+					+	+		+	+					+	+	+		+			+	+			+			
25/9/36	D	+	+	+	+	+		+	+		+		+		+		+	+	+		+			+	+				+	+	
5/10/36	B	+	+	+	+	+		+	+		+				+		+	+	+		+			+	+				+	+	
14/10/36	C			+				+							+		+				+			+	+				+	+	

TABLE II
Fungi on Plates exposed in Grape-fruit Orchards

Date.	Estate.	
8/6/36	D	<i>Fusarium expensum.</i>
19/6/36	D	<i>Botryodiplodia theobromae.</i>
9/7/36	A	<i>Aspergillus niger.</i>
27/7/36	A	<i>Colletotrichum gloeosporioides.</i>
21/8/36	C	<i>Clasterosporium maidicum.</i>
		<i>Nigrospora oryzae.</i>
		<i>Penicillium italicum.</i>
		<i>Basidiomycete spp.</i>
		<i>Acrothecium lunatum.</i>
		<i>Cladosporium herbarum.</i>
		<i>Pectiozia leprogena.</i>
		<i>Spogazania ornata.</i>
		<i>Phomopsis citri.</i>
		<i>Fusarium sambucinum.</i>
		<i>Aspergillus sp.</i>
		<i>Acrothecium sp.</i>
		<i>Rhizopus nigricans.</i>
		<i>Trichoderma lignorum.</i>
		<i>Mucor sp.</i>

the six estates. It would appear that, within limits, the general fungal flora of Trinidad grape-fruit plantations is similar.

Various observers, including Smith (1931) and Taubenhaus (1934), have recorded that the fungi causing stem-end rots of citrus fruit (*Phomopsis citri*

TABLE III
Distribution of Superficial Fungi in Grape-fruit Orchards

	Estate.					
	A.	B.	C.	D.	E.	F.
<i>Pestalozzia leprogena</i>	+	+	+	+	+	+
<i>Penicillium italicum</i>	+	+	+	+	+	+
<i>Fusarium sambucinum</i>	+	+	+	+	+	+
<i>Cladosporium herbarum</i>	+	+	+		+	+
<i>Colletotrichum gloeosporioides</i>	+	+	+	+		
<i>Botryodiplodia theobromae</i>	+	+	+	+	+	+
<i>Trichoderma lignorum</i>	+	+	+	+	+	+
<i>Fusarium expansum</i>	+	+	+	+	+	+
<i>Rhizopus nigricans</i>		+	+	+		+
<i>Mucor</i> spp.	+	+	+	+		+
<i>Clasterosporium maidicum</i>		+	+	+	+	+
<i>Nigrospora oryzae</i>	+	+	+		+	
<i>Phomopsis citri</i>	+	+	+	+	+	
<i>Acrothecium lunatum</i>	+	+	+	+		+
<i>Fusarium lateritium</i>	+	+	+	+		
<i>Papularia sphaerosperma</i>	+				+	+
<i>Pseudoplea trifolii</i>			+	+		
<i>Hormodendrum cladosporioides</i>	+			+	+	
<i>Aspergillus niger</i>	+	+	+			

and *Diplodia natalensis*) are present on the dead wood of citrus trees, and that the removal of dead wood reduces the incidence of these rots. These observations have been confirmed in Trinidad. Other fungi found on dead wood included *Ustilina zonata* and species of *Nectria*, *Eutypella* and *Gnomonia*. The conidia of *Colletotrichum gloeosporioides* were also found, but not in large numbers; the abundant supply of conidia necessary to produce the heavy incidence of latent infections recorded may, however, be derived from any of the numerous alternative hosts of this organism growing in proximity.

2. *Latent infections.* The establishment of latent infections in grape-fruit has been followed from the time of fruit-setting in May until harvesting some nine months later. The results of these investigations are summarized in Tables IV and V. Since it is impossible to determine accurately the age of a grape-fruit, and both the time of setting and the size of fruit in different areas varies considerably, the data is presented in two ways—by weight of fruit (Table IV) and chronologically (Table V). It will be noted that at all times the latent infections of *Colletotrichum gloeosporioides* (*Glomerella cingulata*) far out-number those of any other organism. Latent infections of *Guignardia* sp. are fairly common and those of *Phomopsis citri* occur regularly. Other fungi, including *Fusarium expansum*, possibly occur as latent infections of citrus fruit. The tables of isolations made show that young fruits are heavily infected with *Colletotrichum gloeosporioides* almost as soon as they are set—a condition which persists until the onset of decay in storage. Isolations have

also been made from grape-fruit flowers; *Colletotrichum gloeosporioides* was obtained in culture from the tissues of petals, sepals, receptacles, stigmas and recently set fruits. The evidence available indicates that the development of the fungus is arrested at a very early stage and that infections are

TABLE IV.

Grape-fruit: Isolation of Fungi present as Latent Infections. Under that of Weight of Fruit

Date.	Estate.	No. of fruit.	Mean wt. of fruit (gm.).	Number of pieces of rind plated out.	Colletotrichum gloeosporioides. Total.	Colletotrichum gloeosporioides. Per cent.	Guignardia sp. Total.	Guignardia sp. Per cent.
8/6/36	D	20	0.05	79	13	16	—	—
19/5/36	A	many	0.25	35	10	29	—	—
18/5/36	D	"	0.30	76	55	72	—	—
18/5/36	D	"	0.50	66	3	5	—	—
19/5/36	A	"	0.50	41	23	56	—	—
11/5/36	C	10	1.00	143	39	27	—	—
18/5/36	D	5	1.15	90	24	27	—	—
19/5/36	A	4	1.50	59	9	15	—	—
8/6/36	D	6	8.50	128	31	26	—	—
8/6/36	D	6	16.5	103	10	10	—	—
4/6/36	B	6	17	115	82	71	1	1
11/5/36	C	6	22	93	60	64	—	—
19/6/36	A	6	26	126	24	19	4	3
19/6/36	A	6	40	116	23	20	1	1
13/7/36	B	6	42	116	73	63	37	32
26/6/36	C	6	43	130	81	62	—	—
9/7/36	D	4	45	76	52	68	5	7
27/7/36	A	6	66	120	98	82	14	12
26/6/36	C	6	68	146	42	29	5	3
9/7/36	D	6	88	113	98	87	—	—
13/7/36	B	2	97	38	32	84	10	26
19/8/36	D	6	108	114	59	52	3	3
27/7/36	A	4	175	81	68	84	10	12
27/8/36	D	10	180	250	104	42	12	5
5/8/36	C	6	188	120	33	26	9	7
21/8/36	C	8	191	188	23	12	17	9
19/8/36	D	5	220	103	71	69	4	4
3/9/36	D	10	234	260	138	53	19	7
11/9/36	A	10	255	254	89	35	36	15
25/9/36	D	10	315	240	86	36	17	7

confined to the surface layers of the skin: thus in thin tangential sections of the skin, latent infections were invariably found to be confined to the surface layers. Latent infections may occur over the entire surface of the fruit; they are not noticeably more common at either the stem or the stylar end. As conidia of *Colletotrichum gloeosporioides* are too large to lodge within the stomata, infection of the type described by Kidd and Beaumont (1925) for apple lenticels is precluded.

Dey (1919) has described the direct penetration of the cuticle of beans by *Colletotrichum lindemuthianum*; thereafter the organism persists as mycelium in the epidermal cells. It is a matter of some importance that the infection mechanism and nature of latent infections should be accurately defined if their further development in mature fruit in storage is to be explained: research along these lines is in progress.

Guignardia sp. does not gain entry into fruit as soon as *Colletotrichum gloeosporioides* (*Glomerella cingulata*), and was not found as a latent infection until June, or on fruit smaller than 17 gm. in weight.

TABLE V
Grape-fruit: Isolation of Fungi present as Latent Infections. Chronological Order

Date.	Estate.	No. of fruit.	Mean Wt. of fruit (gm.).	Number of pieces of rind plated out.	<i>Colletotrichum gloeosporioides</i> . Total.	<i>Colletotrichum gloeosporioides</i> . Per cent.	<i>Guignardia</i> sp. Total.	<i>Guignardia</i> sp. Per cent.
11/5/36	C	6	22	93	60	64	—	—
11/5/36	C	10	1	143	39	27	—	—
18/5/36	D	5	1.15	90	24	27	—	—
18/5/36	D	many	0.5	66	3	5	—	—
18/5/36	D	"	0.3	76	55	72	—	—
19/5/36	A	4	1.5	59	9	15	—	—
19/5/36	A	many	0.5	41	23	56	—	—
19/5/36	A	"	0.25	35	10	29	—	—
4/6/36	B	6	17	115	82	71	1	1
8/6/36	D	6	16.5	103	10	10	—	—
8/6/36	D	6	8.5	128	31	26	—	—
8/6/36	D	20	0.05	79	13	16	—	—
19/6/36	A	6	40	116	23	20	1	1
19/6/36	A	6	26	126	24	19	4	3
26/6/36	C	6	68	146	42	29	5	3
26/6/36	C	6	43	130	81	62	—	—
9/7/36	D	4	45	76	52	68	5	7
9/7/36	D	6	88	113	98	87	—	—
13/7/36	B	2	97	38	32	84	10	26
13/7/36	B	6	42	116	73	63	37	32
27/7/36	A	4	175	81	68	84	10	12
27/7/36	A	6	66	120	98	82	14	12
5/8/36	C	6	188	120	33	26	9	7
19/8/36	D	6	108	114	59	52	3	3
19/8/36	D	5	220	103	71	69	4	4
21/8/36	C	8	191	188	23	12	17	9
27/8/36	D	10	180	250	104	42	12	5
3/9/36	D	10	234	260	138	53	19	7
11/9/36	A	10	255	254	89	35	36	15
25/9/36	D	10	315	240	86	36	17	7

VI. FUNGI ASSOCIATED WITH MANGO

The technique already described was found suitable for mangoes. Fruit from three orchards was studied. The organisms obtained from surface washings are indicated in Table VI. By comparing these data with those set out in Table I for grape-fruit, and Table VI for avocado pears, it becomes evident that the organisms listed constitute a common and widely distributed fungal flora of fruit trees in Trinidad.

Colletotrichum gloeosporioides (*Glomerella cingulata*), *Guignardia* sp. and *Phomopsis citri* were present as latent infections, *Colletotrichum gloeosporioides* being the most abundant. Fruits became infected at an early age, e.g. when less than 10 gm. in weight; all fruits above this size were consistently infected. Both lenticels and the tissues lying between them yielded cultures, showing that infection may occur independently of lenticels.

Julie mangoes are generally picked when they are full grown, but still hard and green. At this stage small dark spots less than 1 mm. in diameter are usually visible on the skin both in the tissue between, and in contact with the lenticels. Mycological investigation showed that these are developing

TABLE VI
Mango and Avocado Pear: Superficial Fungi

Organism.	Mango orchard.		Avocado pear orchard.		
	A.	B.	A.	B.	C.
<i>Acrothecium lunatum</i>	+	+	+	+	+
<i>Aspergillus niger</i> and other spp.	+	+	+	+	+
<i>Botryodiplodia theobromae</i>	+		+	+	+
<i>Cladosporium herbarum</i>		+	+	+	+
<i>Clasterosporium maidicum</i>	+			+	+
<i>Colletotrichum gloeosporioides</i>			+	+	
<i>Coprinus</i> sp. and other <i>Basidiomycetes</i>	+	+	+	+	
<i>Fusarium expansum</i>	+		+		
<i>Fusarium lateritium</i>		+	+		
<i>Fusarium sambucinum</i>				+	
<i>Helminthosporium</i> sp.	+	+			
<i>Mucor</i> sp.					+
<i>Neurospora sitophila</i>		+			
<i>Nigrospora oryzae</i>	+	+		+	+
<i>Papularia sphaerosperma</i>				+	
<i>Penicillium italicum</i> and other spp.		+	+		+
<i>Pestalozzia leprogena</i>	+	+	+	+	+
<i>Phomopsis</i> sp. (? <i>citri</i>)	+	+	+	+	+
<i>Pseudoplea trifolii</i>				+	+
<i>Rhizopus nigricans</i>				+	
<i>Spegazzinia ornata</i>		+			
<i>Trichoderma lignorum</i>		+	+	+	+

latent infections of *Colletotrichum gloeosporioides*. As the fruit ripens, these anthracnose spots develop rapidly; at tropical temperatures mangoes become rotten soon after ripening.

Since mangoes suffer severely from blossom-blight caused by *Colletotrichum gloeosporioides*, it is possible that, as in grape-fruit, latent infections are established at this early stage. As a practical issue, the application of a fungicide at the time of flowering has proved effective both in controlling blossom-blight and in reducing latent infections.

VII. FUNGI ASSOCIATED WITH AVOCADO PEAR FRUITS

The fungi found in the surface washings of avocado pears from three localities are given in Table VI; the similarity of this list to that made for the grape-fruit (Table I) and the mango (Table VI) has already been mentioned.

The organisms most commonly present as latent infections are *Colletotrichum gloeosporioides* (*Glomerella cingulata*) and *Guignardia* sp. A *Phomopsis* species indistinguishable from *Phomopsis citri* is found more commonly in the avocado than in either the grape-fruit or the mango. *Botryosphaeria ribis* is found occasionally.

The skin of the avocado pear has a thick-walled epidermis covered by a waxy cuticle, providing a smooth surface on which water does not lie easily. The lenticels appear first as white spots against a green background, but later become suberized and brown. No functional stomata are present on mature fruit.

The latent infections isolated from thirty-three fruits in various stages of maturity are given in Table VII. These were found both at the lenticels and

TABLE VII
Latent Infections of Avocado Pear. Series of Fruit taken by Weight

Weight of fruit (gm.).	Tree.	Number of fruit examined.	<i>Colletotrichum gloeosporioides</i> .	<i>Guignardia</i> sp.	<i>Phomopsis</i> sp. (? <i>citri</i>).	<i>Botryosphaeria ribis</i> .
20	3 and 2	3	9	14	4	—
21-50	3 and 2	6	7	12	6	—
51-100	3 and 2	6	13	35	17	1
112	1	6	3	51	—	—
300	1	6	15	36	—	—
538	3	6	12	18	10	1
	Total	33	59	166	37	2

in the tissues lying between them, some having been established early in the life of the fruit, e.g. in fruit of less than 20 gm. weight (mature fruit may weigh 500 gm.). From the data collected it would appear that there is little increase in infection during the later stages of fruit development.

The large number of *Guignardia* isolations obtained is interesting since this fungus has not so far been observed or recorded as a cause of fruit rotting in avocados.

VIII. FUNGI ASSOCIATED WITH PAPAW, TOMATO AND CACAO

A brief account of the occurrence of latent infections in papaws was given in the first paper of this series. The papaw differs from the grape-fruit, mango and avocado pear in that no isolations of latent infections have so far been obtained from immature fruit. On three occasions series ranging from small recently set fruits to ripe fruits were examined: no cultures could be isolated from fruit less than three-quarters grown (Table VIII). A further series of isolations from four full-grown green or ripening fruits yielded the following cultures:

<i>Colletotrichum gloeosporioides</i>	.	.	.	19
<i>Guignardia</i> sp.	.	.	.	17
<i>Phomopsis</i> (? <i>citri</i>)	.	.	.	5

Latent infections are therefore common in fruit of this type. Observations on the development of fungi on surface-sterilized fruits maintained under aseptic conditions suggest that latent infections may be present in the younger fruits, but that some inhibitory factor prevents their isolation by the technique ordinarily used.

Colletotrichum gloeosporioides and *Phomopsis* (? *citri*) were found commonly as latent infections in both immature and ripening cacao pods. *Phytophthora palmivora*, the cause of 'black-pod disease' of cacao was not found as a latent

TABLE VIII

Latent Infections of the Papaw

Weight (% of weight of ripe fruit).	<i>Colletotrichum</i> <i>gloeosporioides</i> .	<i>Guignardia</i> sp.	<i>Phomopsis</i> sp. (? <i>citri</i>).
0-25	—	—	—
26-50	—	—	—
51-75	—	—	—
76-100	5	14	4

infection. It is possible that this fungus begins to spread as soon as it has gained entry into a cacao pod and has no dormant period.

Latent infections of *Phoma destructiva*, *Guignardia* sp. and *Colletotrichum gloeosporioides* were found in green and in ripening tomato fruits.

IX. RESULTS

As was predicted in the first paper of this series (Baker and Wardlaw, 1937) latent infections of fungi have been found in fruit of all sizes, and it has been shown conclusively that in many instances these latent infections must have been established shortly after the fruit was set. Young grape-fruits less than a week old were shown to be heavily infected with *Colletotrichum gloeosporioides*, and isolations of this fungus were also obtained from all parts of the flowers. There is reason to believe that the large number of spores necessary to produce the heavy infection of *Colletotrichum gloeosporioides* in young fruit is produced on the rotting petals. Similarly, it has been shown that mangoes and avocado pears contain latent infections of several fungi less than two weeks after setting. The actual source of the infection is not known for the avocado pear, but, since mango blossoms are subject to a destructive 'blight' caused by *Colletotrichum gloeosporioides*, the latent infections in young mango fruits are presumed to come from the flowers. The papaw will be the subject of further investigation, since no latent infections could be isolated from fruit less than three-quarters grown, after which they became abundant.

Cultures from the surface washings of fruit and from plates exposed in the orchards showed that there is a large fungal flora common to tropical fruit trees, and, that in the humid tropics such as Trinidad, this flora shows but little seasonal variation.

It has been shown that in citrus fruits the latent infections are very small, and are confined to the surface layers of cells of the skin. They could not be found in stained microtome sections of the skin, and nothing is known at present of their nature, but it is hoped that further work will elucidate this matter.

X. SUMMARY

1. An account is given of previous investigations, conducted both in temperate regions and in the tropics, which have led to the conclusion that certain pathogens gain entry into unripe fruit before the normal time of harvesting. In this way latent infections are produced which are usually invisible when the fruit is picked and only become conspicuous during the later stages of ripening.

2. *Colletotrichum gloeosporioides* (*Glomerella cingulata*), *Guignardia* sp., *Phomopsis citri* and *Dothiorella ribis* occur as latent infections in fruit in Trinidad. Certain other slow growing fungi, which are as yet unidentified, occur occasionally.

3. An account is given of the fungal flora of grape-fruit orchards, and this is compared with those of the mango and the avocado pear. The relative abundance of the different fungi which cause latent infections in tropical fruits is discussed.

4. It is shown that the latent infections of *Colletotrichum gloeosporioides* are established in citrus fruits, mangoes and avocado pears shortly after the fruit is set, the decaying floral parts being in some instances the probable source of infection.

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Possible Action of Phytohormones as Root-determiners

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With twelve Figures in the Text

I. INTRODUCTION

THERE are obviously two possible explanations of the fact that the meristems produced by treating the stems of plants with phytohormones develop into roots and not shoots. The first is that the phytohormones also act as 'root-determiners', i.e. they cause the meristem to develop into a root rather than a shoot. The second is that the meristems so induced, being formed from the cambial regions, are internal and not external, the normal origin of shoots being exogenous and of roots endogenous. Thus Tincker (1937) has suggested that the pressure of the tissues surrounding these new meristems might mould the parenchyma into cylindrical roots.

The following experiments and observations suggest that the phytohormones act as root-determiners. In fact, when the formation of meristems has been stimulated by a dose of hormone, adventitious shoots instead of roots may be formed in the absence of a further supply. Normal axillary buds, or parts of them, have also been observed to grow into roots when hormone was applied to the stem.

2. EXPERIMENTAL TECHNIQUE

It has been shown in various investigations that phytohormones are manufactured in developing buds and leaves, e.g. Went (1929), Thiman and Skoog (1934), and Avery (1935). From these situations they move morphologically downwards. Thus, for example, root development is stimulated at the base of a cutting. In most of the experiments here to be described decapitated plants, as shown in Fig. 1, were used. These plants have a region of stem higher than buds or leaves.

The investigation to be described was part of an attempt to obtain tetraploid Brassicae by somatic doubling and so the plants used were seedlings of kale, *Brassica oleracea*. They were decapitated when they had two to four large leaves. At the same time the axillary buds were removed, but this operation was not always complete and axillary buds were then removed at later

dates. Either the top of the stem or the portion *xy*, shown in Fig. 1, was smeared with the hormone paste.

The hormone used was indole-3-acetic acid in the form of a 1 per cent. paste in anhydrous lanoline. In bright sunlight the paste melts and runs down the stems. This complication was avoided in the later experiments.

The diagrams of the transverse sections, Figs. 2, 3, and 5, were made from enlargements of photomicrographs.

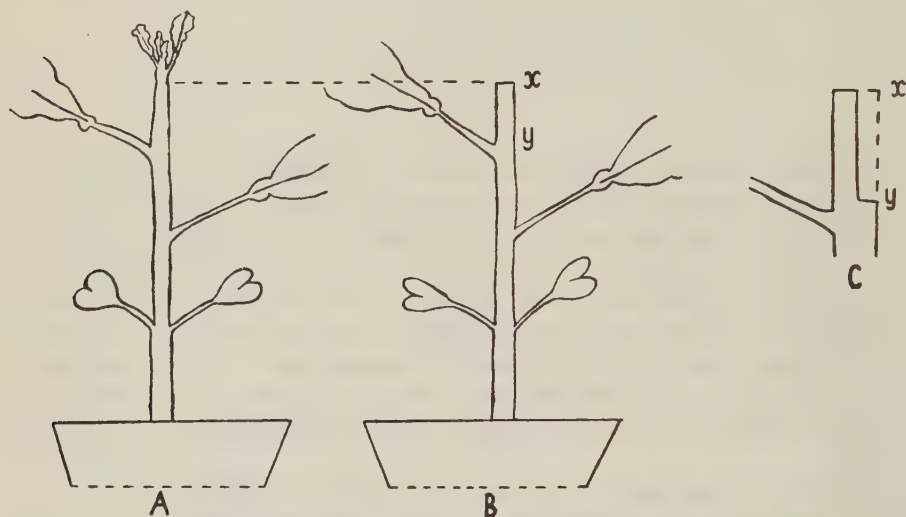


FIG. 1. A. Seedling with two large leaves. B. After decapitation. Part *xy* of the stem is the region on which the hormone smear was placed. C. Top part of the stem enlarged to show the method of longitudinal cutting.

3. PRODUCTION OF ADVENTITIOUS SHOOTS

Ten plants were decapitated and the cut surface given one smear of the hormone paste. In all cases the smear melted and ran down one side of the stem. Only four plants produced adventitious roots on the side of their stems; these roots were removed. Two of these plants later produced on the side of their stems bud-like structures and a number of these grew out into leafy shoots. Besides these four plants there were also two which produced adventitious shoots from the sides of their stems without previously forming adventitious roots.

It was also observed in one plant that, when the leafy shoots had been growing for about four weeks, in two or three places on the old stem below a leafy shoot there was a renewed production of adventitious roots. Fig. 4 is a photograph of this plant; all the shoots except the one photographed had been removed as cuttings to see if any were tetraploid. This production of a second batch of adventitious roots has also been observed in five plants of later experiments after leafy shoots had grown.

As controls there were five plants which were decapitated and another five which were decapitated and also had the cut surface smeared with pure lanoline. None of the control plants produced adventitious roots and two only of them produced callus buds. These callus buds were on the cut surface and not from the side of the stems. At least eighty kale plants had been decapitated and not treated with hormone pastes in earlier experiments to obtain callus buds. In no case had the production of adventitious roots been observed and callus buds were always formed on the cut surface only and never from the side of the stem.

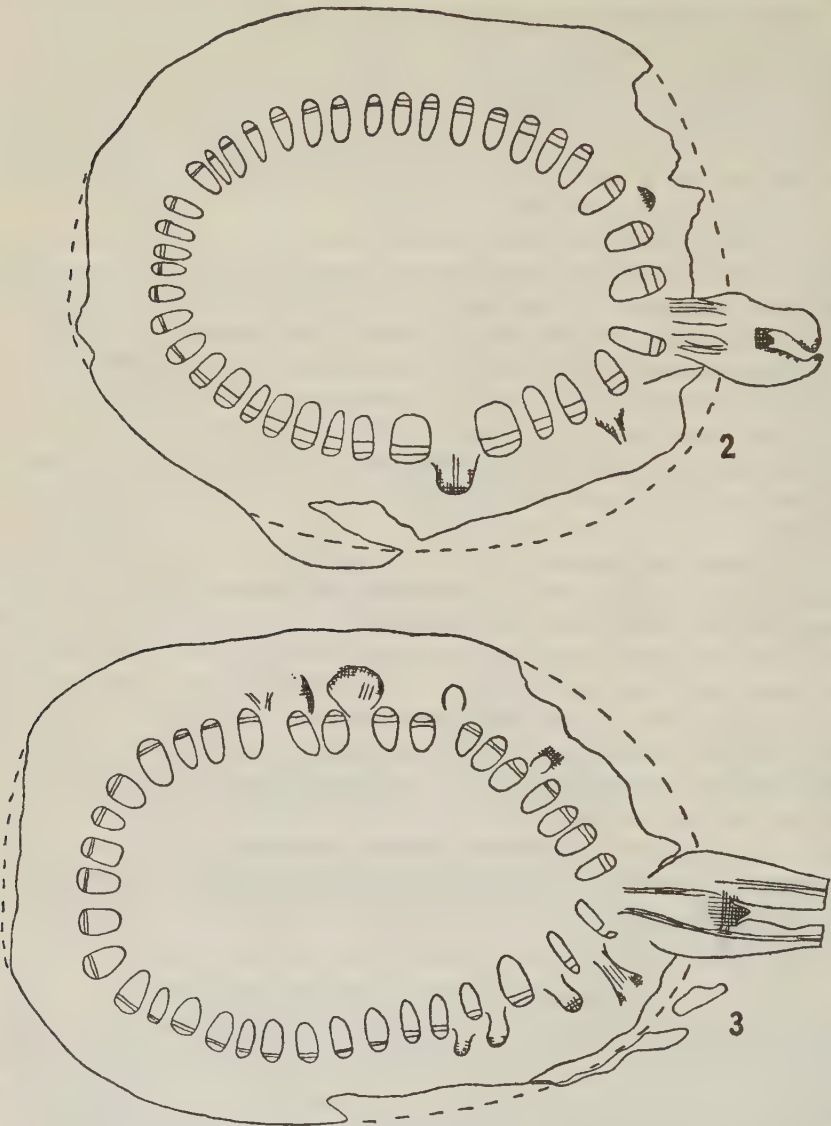
Another set of plants was decapitated and one side of their stems, region *xy* in Fig. 1, was smeared with the hormone paste. The stem of one of these plants, which produced adventitious shoot buds without a previous production of roots, was fixed when the buds were still small, embedded in wax, and sectioned transversely. Figs. 2 and 3 are diagrams of two of these sections showing two different shoot buds. The sections show that the buds have an origin from near the vascular bundles. There could also be seen in the sections small meristems which had not reached the exterior. It was presumably from such meristems that the second batch of adventitious roots was produced.

The simplest explanation of the preceding observations is that the phytohormone has two effects; first it promotes the formation of adventitious meristems and then it acts as a root-determiner on these meristems. Thus the developing meristems become roots instead of shoots at the beginning of an experiment only, i.e. when there is a supply of hormone present. The renewed production of roots may have been due to the manufacture of a hormone by the developing adventitious shoots. An alternative explanation is that there are produced two types of meristems, root and shoot meristems, and that the root meristems only grow when there is a supply of hormone present, the shoot meristems when hormone is absent. If there are produced two types of meristems, it is to be expected that they will be produced from different tissues; otherwise there is no reason for supposing that there is more than one type of meristem.

4. EXPERIMENTS WITH CUT AND SCRAPED STEMS

The fact that adventitious buds can originate from meristems formed from tissues near the vascular bundles was very clearly shown in four plants. These plants were decapitated and one side of their stems smeared with the hormone paste. After one week the hormone paste was removed and at the same time the sides of the stems were scraped until the vascular bundles could be seen. These plants formed adventitious buds on the scraped parts and these buds could not have come from any tissue but one very near the vascular bundles. Fig. 6 is a photograph of one of these plants. Later one of these plants formed adventitious roots on the scraped part of the old stem beneath the now leafy buds. These roots could be seen to originate from the same regions as the

adventitious shoots. They also had an external origin because of the scraping of the stem. There is thus no reason to believe that the place of origin decides



FIGS. 2 and 3. Diagrams of transverse sections of stems showing the origin of two different adventitious shoot buds. The broken line shows the hypothetical position of the epidermis where this and part of the outer cortex have been lost. Vascular bundles are drawn in outline. Meristems are shown as small squares.

why some meristems become roots and others shoots. Also the pressure of surrounding tissues does not appear to be necessary for the growth of meristems as roots.

Similar conclusions to those in the last paragraph were also suggested by the cut-stem experiments. In these experiments a half-cylinder of the top part of the stem, as shown in diagram c of Fig. 1, was removed from the decapitated plants. The cut surface was then smeared with the hormone paste. It can be seen in Fig. 5, which is a diagram of a transverse section through one of these stems one week after decapitation, that some of the meristems produced are not internal to the whole cortex and epidermis but only to a small part of the inner cortex at the most. The plants with cut stems were treated in three ways as is shown in the table.

Plants 21-4 never produced any outgrowths. Plants 6-20, with the exception of plants 10 and 20, all produced adventitious roots from the cut surface before 19. There is thus no need for the pressure of surrounding tissues for adventitious meristems to grow into roots.

Plants 17, 18, and 19 produced shoot buds when the hormone paste was removed. Because of the cut stems it was possible to see that these buds were produced from meristems very close to others which had previously grown into roots. Plants 17 and 18 also formed a second batch of adventitious roots



FIG. 4. Photograph of plant with a second batch of adventitious roots formed below a leafy adventitious shoot.

Treatments of Plants with Cut Stems

Plants.	First Treatment.	Second Treatment.
6, 7, 8, 9,	Cut 22·8, and hormone	New smears of hormone on
10.	paste applied.	7·9, 17·9, and 24·9.
16, 17, 18,	Cut 22·8, and hormone	Hormone smear removed on
19, 20.	paste applied. New	1·9, and no more applied.
	smear on 28·8.	
21, 22, 23,	Cut 22·8, and pure	Lanoline removed.
24.	lanoline applied.	

when the adventitious buds had developed into shoots having about two large leaves. These roots could also be seen to come from meristems close to those meristems which had grown into shoots. There thus seems to be very little doubt that there are not two different tissues producing two different types of meristems.

The continued application of hormone paste to the stems of plants 6-10 caused the stems of these plants to rot. Plant 6 was an exception and the stem of this plant was still producing adventitious roots when the adventitious buds

on plants 17, 18, and 19 could be recognized. Thus in this one plant in the presence of a continuous supply of hormone, meristems continued to grow as roots and no shoots were formed. Fig. 7 is a photograph of plant 18 when the buds had grown out into leafy shoots. When these shoots were removed

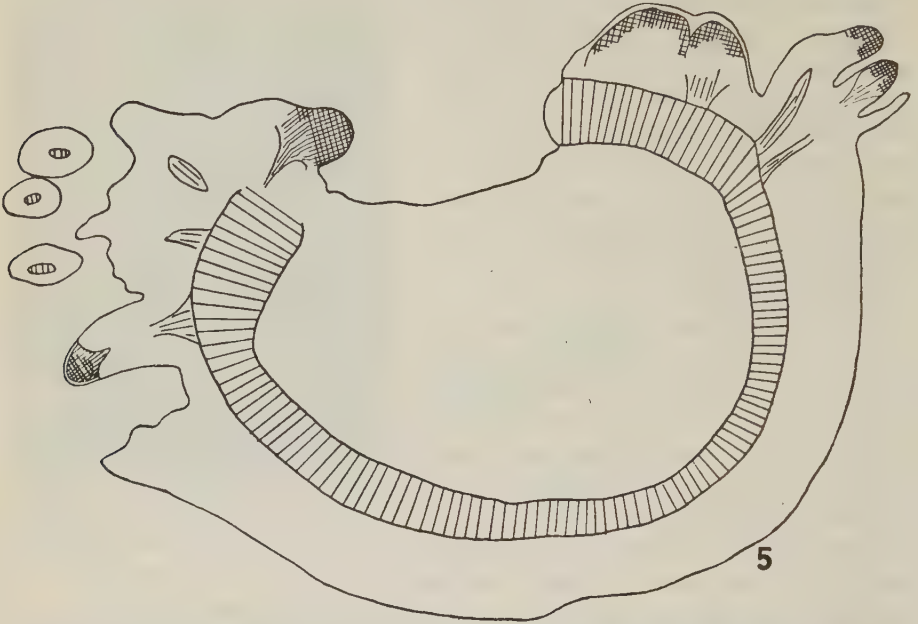


FIG. 5. Diagram of transverse section of treated half-cut stem, one week after decapitation. Xylem of vascular bundles now continuous and shown by shading. Meristems shown by small squares.

as cuttings, more adventitious shoots were formed. These may have developed from undifferentiated meristems, or from shoot meristems which were kept from growing by the inhibiting effect of the leafy shoots. When this second batch of leafy shoots had grown for some time a third batch of adventitious roots was produced.

5. GROWTH OF AXILLARY BUDS

In the autumn of 1937 a number of plants were treated with growth-hormone paste. These plants were not decapitated but a half-cylinder of the stem below the terminal bud was removed (in the same position as that removed from the decapitated plants described above). The cut surface was smeared with the hormone paste. These plants formed adventitious roots very slowly in comparison with those plants treated earlier in the year. In several cases it was noticed that roots were growing from the axils of the cotyledons. These roots or root-initials could be very easily recognized by their white colour. Normal axillary buds are green or purplish in colour. In no case had growth-hormone paste been applied to these axillary buds but always to the

stem about an inch above these places. In all cases the cotyledons had dropped off. Samples of these stems were taken and sectioned longitudinally. In many untreated kale seedlings roots in the axils of the cotyledons have never been observed.



FIG. 6.



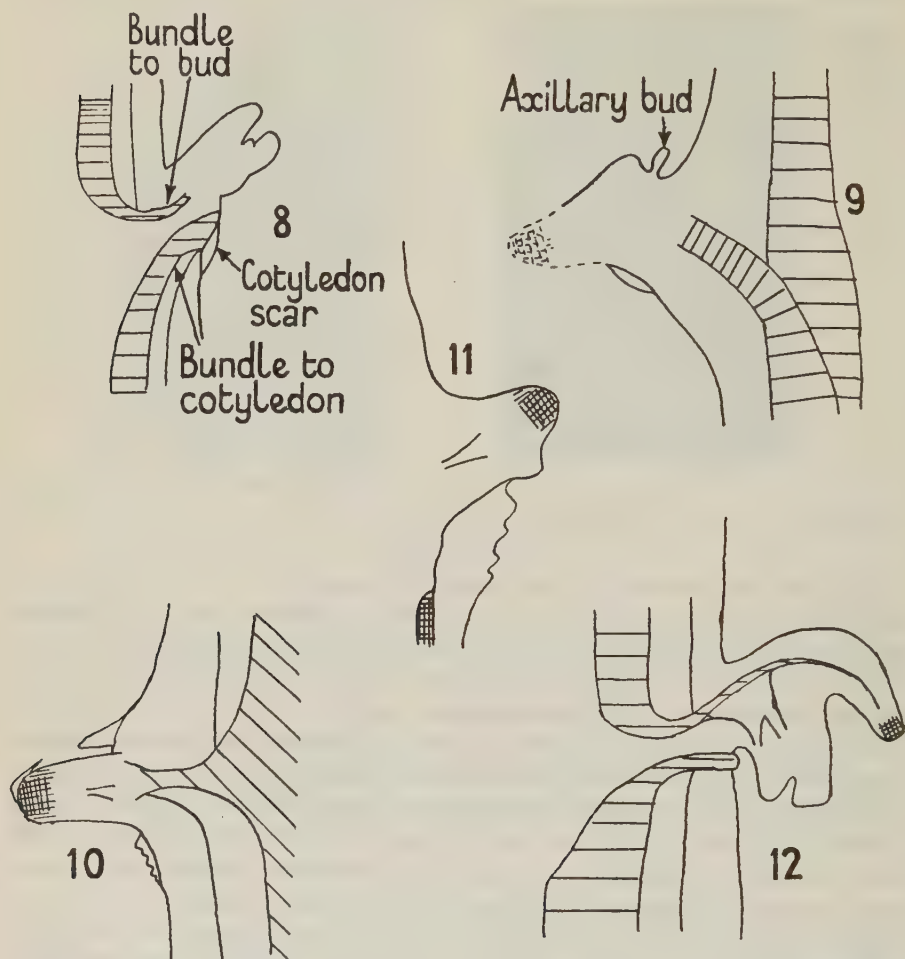
FIG. 7.

FIGS. 6 and 7. Fig. 6. Scraped stem with adventitious shoot buds. Fig. 7. Half-cut stem with adventitious shoot buds.

Figs. 8–12 are sections through stems of treated plants in the region of the axils of the cotyledons. Fig. 8 shows a normal axillary bud. The vascular bundle to the cotyledon and the scar formed where the cotyledon has fallen off can also be seen. Fig. 9 shows a root which has developed not from the axillary meristem but from an adventitious meristem formed on the cotyledon scar. The broken lines show the root-tip which could only be seen in other sections. Fig. 10 also shows a root which has probably grown from an adventitious meristem since there is dead tissue both below and above the outgrowth. Fig. 11, on the other hand, shows a root meristem which has certainly been formed from the normal axillary meristem. Only the top part has become a root meristem and this part has grown faster than the bottom part. There can also be seen in this section an adventitious meristem below the cotyledon scar. Fig. 12 shows one leaf initial of an axillary bud growing as a root. A similar development of the axillary bud of the other cotyledon had occurred. The greater growth of the top leaf initial as a root meristem has forced the rest of the bud downwards. There was no doubt that it was a leaf initial which had been converted into a root. As can be seen in the figure its vascular supply is connected with that of the rest of the bud. The bundle to the cotyledon can also be seen.

In kale seedlings the axillary meristems grow after a time into small buds which cease to grow any larger under normal conditions when they are about $\frac{1}{2}$ inch long. The above results can be explained as being due to the root-

determining action of the growth hormone transported down the stem and acting on different stages of the growth of these axillary meristems. If the hormone acts on a very early stage the whole meristem becomes a root; at a later stage only one leaf initial grows into a root. The supposed downwards



FIGS. 8-12. Longitudinal sections through axils of cotyledons; for description see text. Xylem of vascular bundles shown by shading. Meristems shown by small squares.

transport of the hormone would be helped by the cutting of the stem so that it was in direct contact with the phloem and may be connected with the slow production of adventitious roots. It is probably significant that the parts of the axillary buds which became root meristems were the upper parts which were those nearest to the supply. Taken together with the observations on adventitious meristems these observations on the growth of axillary meristems suggest very strongly that the hormone has a root-determining effect.

6. DISCUSSION

Williams (1937) has reported that preliminary experiments in *Selaginella* show that the presence or absence of hetero-auxin is an effective factor in determining whether an angle meristem shall develop as a rhizophore or as a leafy shoot. This conclusion is similar to the root-determining action suggested in this paper.

Zimmerman, Crocker, and Hitchcock (1933) have caused plants to produce adventitious roots on their stems by treating them with carbon monoxide. This is not contrary to the above conclusions because in their experiments the roots were produced in regions of stems morphologically inferior to leaves and developing buds and these would act as suppliers of hormone.

If the hormones do act as root-determiners, then the question arises how they have this effect. It has been shown by Snow (1935) that hormones can stimulate cambial growth. Both cambial growth and the growth of a root-tip meristem agree in that most new cell walls are produced in one direction and parallel to each other. If the new cell walls are formed at right angles to the hormone gradient (assumed in normal stems to be along radii from the phloem), then cambial growth stimulation and root-determining effect can be correlated. There is a little additional evidence for this suggestion. The adventitious roots formed by smearing the stems with growth-hormone paste are much thicker than those produced later when the adventitious shoots supply the hormone from the inside only and, it is suggested, to the meristem in a longitudinal direction only. Similarly it was found that the adventitious roots produced on non-decapitated plants were thick in the parts near the stem (i.e. where they had been surrounded by growth hormone) but became much thinner and normal in size as they grew away from the stem and the hormone was presumably only supplied to the adventitious root via its vascular system. On the other hand the effect may be a very different one. Thus the root meristems produced in the axils of the cotyledons were very easily seen to be different from the normal shoot buds by their white colour. Also it is possible to grow root-tips for very long periods in a culture medium which does not contain hormone, and after many subculturings the meristems still continue to grow as roots.

SUMMARY

1. The stems of decapitated kale plants were treated with paste containing indole-3-acetic acid. Adventitious shoots in addition to roots were produced. These shoots are produced at a later date than the roots or when the supply of hormone is removed.

2. The production of a second batch of adventitious roots was observed when the adventitious shoots had grown for some time.

3. Both adventitious roots and shoots are formed from meristems produced near the vascular bundles.

4. Buds or parts of buds in the axils of the cotyledons were found to develop as roots when the hormone was applied to the stem at a higher level.
5. It is suggested that the most satisfactory explanation of the results is that the hormone has two effects: first it promotes the formation of meristems and then has a root-determining effect on these meristems. The growth of the second batch of adventitious roots (see paragraph 2 of summary) is explained as being due to the production of a hormone by the leafy shoots.
6. It is also shown that an internal origin is not necessary for roots.

APPENDIX

Since this paper was sent to the press Stoughton and Plant (*Nature*, 1938, 142, 293-294) have described their experiments with root cuttings of sea-kale (*Crambe maritima*). Their conclusion is similar to the one reached in this paper. They state: 'From these experiments it is suggested that whatever other substances may be concerned in bud and root production as proposed by Went, not only the subsequent growth but also the initial differentiation of meristematic tissue is determined, at least in part, by the local concentration of growth substance.'

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The Formation of Proteins in Leaves in Light of Different Quality

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A BRIEF reference to the work of one of the authors (1933, 1935, 1937) on the formation of carbohydrates in leaves exposed to different lights is necessary in order to understand the object of this investigation and the results discussed below. It was first shown that leaves exposed to an ordinary electric lamp contained less carbohydrates than those exposed to diffuse daylight of equal total intensity. The differences in the carbohydrate content of the leaves in the two lights were attributed to the differences in their spectral qualities. It was later shown that low photosynthetic activity in artificial lights was due to the lower proportion of the blue-violet rays present in them. Using lamps differing in the proportion of blue-violet rays it was shown that the photosynthetic activity decreased as the ratio of the intensity of red to blue-violet rays increased. The quantity of carbohydrates present in the leaves in the different lights were in the order: sunlight > arc lamp > 'daylight' lamp > ordinary electric lamp. Thus in white light the relative amounts of radiation of different wave-length are important in assimilation.

Working with red light (6,200–7,000), blue-violet light (4,000–4,700), and daylight of equal intensities, it was found that photosynthetic activity was highest in white light, low in red light, and still lower in blue-violet light. It was thus established that the red region and the blue-violet region were both necessary for the normal photosynthetic activity, and that even with white light the assimilation depends on the distribution of radiation of different wave-length.

As the photosynthetic activity is measured by determining the amounts of carbohydrates present in the leaves after exposure to each quality of light, the results as such may not give a true picture of the assimilatory activity of the leaves under each condition, as the determinations are of the carbohydrates accumulated but not of the carbohydrates synthesized. It is

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reasonable to assume that some of them may have been utilized in the synthesis of proteins in the leaves. It is therefore likely that the lesser amounts of carbohydrates found in leaves in artificial sources of light may not be due to a lower photosynthetic activity but to a more or less rapid utilization of carbohydrates in the synthesis of proteins. It seemed therefore advisable to test this point by determining the quantity of proteins formed in leaves under similar conditions of experimentation employed in the previous work.

The methods of exposure, the sources of lights used, and the species selected for work were the same as those employed in the work mentioned above. The plants used were *Helianthus annuus*, *Raphanus sativus*, *Nicotiana Tabacum*, *Ricinus communis*, *Abutilon asiaticum*, and *Tropaeolum majus*. The sources of light employed were daylight, 1,500-watt ordinary electric lamp, 1,500-watt 'daylight' lamp, and a carbon-arc lamp. The red (6,200–7,000) and blue-violet (4,000–5,000) lights were obtained by using a carbon-arc lamp and daylight. The intensities were made equal in all cases by the method employed in previous investigations.

Potted plants were used. They were kept in the dark for seventy-two hours before exposure to lights. Five replicated experiments were performed in each set. The leaves taken for analysis were just fully grown; old leaves were avoided.

METHODS USED IN THE PREPARATION OF THE MATERIAL FOR ANALYSIS

In studying changes in plant metabolism under different conditions it is often necessary to preserve the material for future analysis. From the review of literature, drying seems to be one of the most reliable methods for preservation when the plant tissue is not of the acid type. It is, however, necessary that proteolytic changes occurring in the leaves during the process of preparation of the material should be reduced to a minimum in order that the results may be expressive of the changes in leaves on illumination. Chibnall (1922) has shown that considerable changes occur in the nitrogenous constituents of the leaves of runner beans at room or low temperatures. In this case enzyme action is not precluded and proteolysis sets in. Osborne and Wakeman (1920) point out that there is very slight protein change in leaves properly dried at 60° C. as compared with fresh leaves used for analysis, whereas at lower temperatures considerable proteolysis occurs. Link and Schulze (1924), who have made a thorough investigation of the effects of desiccation on various tissues, state that *each tissue represents a separate case and no general conclusions applying to all types of tissue can be drawn*. However, higher temperatures, 80°–98°, gave a diminution of soluble N by coagulation, whereas lower temperatures, 32°–45°, gave higher proportions of this fraction due to autolysis. Thus the general effect is a decrease of soluble N by coagulation or desiccation. Mulay (1931) suggests that proteolytic changes occurring at 55° C. are negligible when drying is done in a hot-air oven. It is thus evident that

considerable variation of opinion exists as to the effect of temperature in drying, this variation being mainly due to differences in the material employed.

In our studies an experimental comparison was made between the drying method used by Mulay (1931) and the one suggested by Link and Schulze (1929) and improved upon by Pearsall and Wright (1929). The latter workers dried material at 95° C. for half an hour and then at 60° C. to constant weight; the first half-hour's drying being employed to kill enzymes. The results indicated that the values for the total water-soluble organic N and the insoluble protein N were the same by these two methods, but there were differences in the relative distribution of various soluble fractions, such as amino N, polypeptide N, &c. As in the present investigation the changes in the relative distribution of soluble fractions do not affect the general trend of the results, the method used by Mulay (1931) is therefore considered to be satisfactory.

Due to these differences found on analysis the total water-soluble protein N, polypeptide and diamino N, monoamino N, and amide N are not given or considered separately, but are added together and termed 'water-soluble organic N' in the results given below (Tables I to VI). In this investigation it will be seen that inorganic N is not considered. In each experiment the values of the total soluble organic N found in the leaves before exposure to each light are subtracted from those found in the leaves after exposure, unless otherwise stated. The residual organic nitrogen is generally taken as insoluble protein N. This was determined, but is not given in the results, as only the mobile forms of proteins are important. The results showed no increase in the insoluble protein nitrogen after exposure to light, but there was marked increase in the water-soluble organic nitrogen including protein, polypeptides, and amino acids after such exposure, indicating that they are synthesized during the period of exposure.

Extraction with water.

One gramme of the powdered material is taken and mixed with 50 c.c. of cold water in a 100-c.c. conical flask. The flask is shaken on a mechanical shaker for two hours, centrifugalized, and decanted off. The leaf material is further mixed with water, shaken, and centrifugalized, and the combined water extracts made up to 250 c.c. Fifty cubic centimetres of this solution are taken for determination of total nitrogen by means of Gunning's method. The result is termed *A*.

One hundred and fifty cubic centimetres of the remaining extract are then taken in a beaker and the water-soluble proteins are precipitated with colloidal ferric hydroxide after bringing the pH of the extract to 4. The extract is centrifugalized and the precipitate washed twice with distilled water and the washings added to the mother liquid. One-third of the resulting liquid is then taken and the total nitrogen is determined. This is termed *B*. The difference between *A* and *B* gives the total water-soluble protein nitrogen. The

remaining two-thirds of the filtrate, after precipitation with ferric hydroxide, is treated with 20 per cent. phosphotungstic acid and is allowed to stand for a few hours and then filtered. Half of the filtrate is taken for the estimation of total nitrogen. This is termed *C*. The difference between *B* and *C* gives the nitrogen of polypeptides and diamino acids, following Lincoln and Mulay (1929).

The remaining half of the filtrate, after precipitation with phosphotungstic acid, is mixed with an equal quantity of 8 per cent. hydrochloric acid and boiled for two hours under a reflux condenser to hydrolyse the amides. The solution is made alkaline with magnesium oxide and ammonia is distilled off. The nitrogen thus obtained is the amide nitrogen.

The residue remaining after extraction is estimated for total nitrogen to give the residual organic insoluble nitrogen.

Another gramme of the dry sample is shaken up with 20 c.c. of water, centrifugalized, washed as before, and then made up to 50 c.c. Monoamino acids are determined in this extract by Van Slyke's method.

INVESTIGATION

It was necessary to study the protein contents of leaves during photosynthesis in white lights derived from different sources and to see how they affect the conclusions reached earlier regarding the effect of lights of different spectral intensities on the formation of carbohydrates in leaves. Daylight, a carbon-arc lamp, a 'daylight' lamp, and an ordinary gas-filled electric lamp were used as sources of light.

The results of soluble total organic nitrogen (including soluble protein N) are given below in Table I. The formation of proteins in the leaves of *Helianthus* and *Ricinus* is highest in the 'daylight' bulb, medium in the ordinary electric, and low in daylight. Similarly, formation of proteins in the leaves of *Abutilon asiaticum* is highest in the carbon-arc lamp. Comparing the results of soluble total organic N in leaves of *Helianthus annuus* exposed to a carbon-arc and a 'daylight' lamp, it is found that the formation of proteins in the former light is greater than in the latter.

The results of the soluble organic N formed in these lights were examined by Student's method to see if the differences are statistically significant; the results are given below.

The protein formation in these lights is in the order: carbon arc > 'daylight' lamp > ordinary electric lamp > daylight; while the carbohydrate formation in the same lights was in the order: daylight > carbon-arc > 'daylight' lamp > ordinary electric lamp.

The results obtained both here and before show increased formation of proteins with low photosynthetic activity in the ordinary electric light, while in the sunlight there is a decreased formation of proteins with high photosynthetic activity. It therefore appears that more carbohydrates are actually formed in the electric light than are shown by analysis, the rest being utilized

in the protein synthesis. There is, however, low protein synthetic activity in daylight where carbohydrates are found in larger amounts. The only explanation is that in daylight, with its high photosynthetic activity, the process of protein synthesis is depressed, very probably as a result of the accumulation

TABLE I

Water-soluble Total Organic N (mg. per 100 gm. dry wt.) produced in Leaves after Exposure for 5 Hours)

<i>Ricinis communis</i>						
	Expt. 1.	Expt. 2.	Expt. 3.	Expt. 4.	Expt. 5.	Mean.
'Daylight' lamp	618	522	614	527	444	545
Ordinary electric lamp	298	273	383	351	222	325
Daylight	279	202	193	205	150	206
<i>Helianthus annuus</i>						
'Daylight' lamp	364	307	420	271	—	340
Ordinary electric lamp	320	244	326	145	—	259
Daylight	108	135	197	61	—	143
<i>Abutilon asiaticum</i>						
Carbon-arc	406	656	395	572	437	493
Ordinary electric lamp	247	285	324	291	306	290
Daylight	236	252	237	327	290	205
<i>Helianthus annuus</i>						
Carbon-arc	206	50	241	523	314	267
'Daylight' lamp	120	59	131	390	212	182

TABLE II

	<i>Helianthus.</i>	<i>Abutilon.</i>	<i>Ricinus.</i>
Ordinary electric <i>v.</i> sunlight	Significant	Not significant	Significant
Daylight lamp <i>v.</i> ordinary electric	Significant		Highly significant
Carbon arc <i>v.</i> daylight lamp		Significant	
Carbon arc <i>v.</i> ordinary electric		Significant	

of carbohydrates, while in the ordinary electric light low photosynthetic activity does not interfere with the process of formation of proteins and most of the carbohydrates that are produced are utilized in that process. This explanation is in accordance with the results of the quantities of proteins present in the leaves exposed to the carbon-arc and the 'daylight' lamp, where, on account of the presence of higher proportion of blue-violet rays, the photosynthetic activity is higher than in the ordinary electric light accordingly more proteins are formed in the carbon-arc and in the 'daylight' lamp than in ordinary electric light, as carbohydrates formed are higher in the former than in the latter. At the same time the formation of carbohydrates in the carbon-arc and the 'daylight' lamp is not so high as to interfere with the process of protein synthesis as is the case with daylight.

In order to test the above conclusions, a set of experiments was arranged to discover if there is a greater formation of proteins in electric light than in

other lights when the supply of carbohydrates is not limiting the process. A batch of potted plants of *Tropaeolum majus* was first kept in the dark for seventy-two hours, as usual. They were then *all* exposed to direct sunlight for three hours in the morning in order to produce sufficient amounts of carbohydrates in the leaves. After three hours they were divided into five groups. One group was allowed to remain in daylight, the second one was exposed for three hours to ordinary electric light, the third one to a carbon-arc lamp, the fourth to a 'daylight' lamp, and the fifth was taken for protein analysis. A group of plants was also taken for analysis before the first exposure of three hours to sunlight. After the additional exposure of three hours to each light the leaves and petioles of the four groups were taken for analysis of total organic nitrogen. The results of the total soluble organic nitrogen present and formed in the exposure of three hours to each light are given in Table III in the case of the five replicated experiments. In the former set of experiments the values of soluble organic nitrogen are for six hours, while in this experiment they are for three hours.

TABLE III

Water-soluble Organic N (mg. per 100 gm. dry wt.) of Leaves Before and After Exposure to Different Lights

<i>Tropaeolum majus</i>							Increase in soluble organic N in 3 hours.
Exposure.	Expt. 1.	Expt. 2.	Expt. 3.	Expt. 4.	Expt. 5.	Mean.	
Dark	234	193	211	305	195	227	
Daylight (3 hours)	354	331	247	338	301	314	87
Daylight	406	331	358	348	412	371	57
Carbon-arc lamp	400	275	422	345	382	365	51
'Daylight' lamp	449	278	408	314	314	352	38
Ordinary electric lamp	506	473	679	381	411	490	176

The results show that greater formation of proteins occurs in electric light than in daylight when the supply of carbohydrates is not limiting the process. During the first three hours' exposure to sunlight the carbohydrates accumulate, and during the second exposure of three hours to ordinary electric light the process of protein synthesis goes on at a higher rate than in other lights as the photosynthetic process is depressed due to the deficiency of blue-violet rays. As some carbohydrates had already accumulated in the first three hours' exposure to daylight, a part of them, along with those that are formed in the second three hours' exposure to electric light, must have been utilized for the formation of proteins. But the results also suggest that enough carbohydrates have accumulated in three hours in sunlight to prevent protein synthesis

going on at its maximum rate. The amounts of proteins formed in the second exposure of three hours in daylight, carbon-arc lamp, and 'daylight' lamp are more or less the same, firstly because enough photosynthetic products have accumulated in the first exposure of three hours to daylight, and secondly the photosynthetic process proceeds at a higher rate in these lights than in the ordinary electric light during the second three hours' exposure.

A determination was also made of the proteins formed in electric light in the leaves of the same species without previous exposure to daylight to discover if greater amounts of proteins are formed in leaves by increasing the supply of carbohydrates than in the leaves where the additional supply of carbohydrates is not increased. The results (Table IV) suggest that enough carbohydrates have accumulated in three hours in daylight to depress protein synthesis.

TABLE IV

Water-soluble Organic N (mg. in 100 gm. dry wt.) formed in Leaves of Tropaeolum majus in Ordinary Electric Light with and without a previous three hours Exposure to Daylight

		Expt. 1.	Expt. 2.	Expt. 3.	Expt. 4.	Expt. 5.	Mean.
Without exposure	A	188	206	343	382	235	271
	B	232	370	390	216	296	281
With exposure		304	284	864	86	220	351

Proteins formed during exposure to monochromatic blue-violet and red lights.

Dastur and Mehta (1933) found that more carbohydrate was formed in leaves in red light than in blue-violet, while the highest amount was formed in white light (daylight) of equal intensity. The order was: daylight > red light > blue-violet. For the various coloured lights sunlight was used as a source since the total intensity of blue-violet in ordinary electric light was too low for the process to go on. To make the total intensities of the two monochromatic lights equal to the white light, they employed an indirect method by which the total transmission of white and red lights was made equal to that of the blue-violet light, the total transmission in the latter being 23.9 per cent. Thus the total intensity of each light employed was very low.

In view of the results obtained with carbohydrates, with the two monochromatic lights and with white light it was of interest to determine the proteins formed in blue-violet and red lights. The same method was adopted to obtain blue-violet and red light.

Although there is an indication of slightly greater formation of proteins in the blue-violet than in the red light, the differences between the two are not statistically significant, the value of P exceeding 0.05. In white light the formation of proteins in leaves of *Helianthus* is greater than in the other two monochromatic lights. As the photosynthetic activity is low in monochromatic light the synthesis of proteins is also correspondingly low. The results of carbohydrate analysis of the leaves in the monochromatic lights showed

that the photosynthetic activity was higher in the red than in the blue-violet, while the proteins formed in the two cases were equal. It therefore appears that some amount of the carbohydrates formed in the blue-violet and red light are utilized in protein synthesis, a higher surplus of carbohydrates being

TABLE V

Water-soluble Organic N (mg. in 100 gm. dry wt.) formed in Leaves in Red and Blue-violet Light of Low Intensity

<i>Helianthus annuus</i>							
	Expt. 1.	Expt. 2.	Expt. 3.	Expt. 4.	Expt. 5.	Mean.	P.
Blue-violet . .	26	63	—	54	84	56	0.2 P 0.1
Red . . .	51	49	—	47	61	43	
Daylight . .	108	135	—	197	61	143	
<i>Tropaeolum majus</i>							
Blue-violet . .	131	85	223	177	181	159	0.1 P 0.05
Red . . .	106	148	84	82	106	105	
<i>Raphanus sativus</i>							
Blue-violet . .	37	49	25	68	88	53	0.7 P 0.6
Red . . .	56	46	21	97	6	45	

left in the red light than in the blue-violet light as a result of the greater photosynthetic activity of the former. If this is so, an increase in the total intensities of the red and blue-violet lights beyond that employed in the above experiment should give more proteins in the blue-violet light than in the red, as it is possible that carbohydrate supply may be more strongly limiting in the blue-violet light than in the red. By increasing the total intensity of the two lights carbohydrate supply will increase in the blue light, and there will therefore be greater protein synthesis as in the electric light; in the red light, however, a further increase in carbohydrates may not increase the rate of protein synthesis, as is the case in daylight.

The carbon-arc was used as a source of light, and a much higher intensity of monochromatic blue light was obtained than before. The intensity of the red light was made equal to that of the blue-violet light by interposing glass plates, as before (1935). The results of total protein N formed in the two monochromatic lights in leaves of four species are given in the Table VI.

The results show that greater amounts of soluble organic N, including water-soluble proteins, are formed in the leaves in the blue-violet light than in the red light. The differences between the means of total water-soluble organic N contents in blue-violet light and red light are statistically significant, with P 0.01.

On comparing the results in Tables V and VI it will be seen that the higher intensity of the blue-violet light has resulted in the formation of greater amounts of proteins in the leaves of the three species. The differences in the mean organic N contents of leaves exposed to a low and high intensity of blue-violet light are found to be statistically significant in these species. This

is not so in the case of the leaves exposed to low and high intensity of red light where the differences between the mean total organic nitrogen contents of the leaves are not statistically significant. By increasing the intensity of red light the rate of protein synthesis is not increased though the rate of photosynthesis may have been increased.

TABLE VI

Water-soluble Organic N (mg. in 100 gm. dry wt.) formed in Leaves in Lights of Equal Intensity. Intensity higher than in Table V

<i>Tropaeolum majus</i>						
	Expt. 1.	Expt. 2.	Expt. 3.	Expt. 4.	Expt. 5.	Mean.
Blue-violet	316	251	333	193	304	279
Red	147	200	92	128	160	145
						P 0.01
<i>Raphanus sativus</i>						
Blue-violet	102	109	72	100	134	105
Red	72	106	31	39	29	55
						P 0.05
<i>Helianthus annuus</i>						
Blue-violet	112	118	191	100	144	133
Red	28	51	63	85	57	57
						P 0.01
<i>Nicotiana Tabacum</i>						
Blue-violet	160	120	136	178	160	151
Red	60	38	24	113	68	60

CONCLUSIONS

The results obtained above for the formation of proteins during photosynthesis in leaves exposed to white lights of different spectral quality and to monochromatic light do not in any way affect the general conclusions reached in the previous investigations as to the importance of blue-violet rays in the photosynthetic process. When the ratio of the intensity of red to that of blue-violet rays is high, as in the case of ordinary electric light, the photosynthetic production of carbohydrates is depressed and the formation of proteins, although not interfered with, is limited by the carbohydrate supply. When this ratio is low, as in daylight, the process of carbohydrate synthesis goes on at its maximum rate and the process of protein synthesis is low, due to the large accumulations of the products of photosynthesis. With an intermediate ratio of red to blue-violet rays, as in the case of the carbon-arc, both the processes go on without any interference and the synthesis of proteins continues at a greater rate than in the electric light.

The results obtained with the two monochromatic lights also support the same conclusions. With low intensity of red or of blue-violet rays the low rates of carbohydrate synthesis do not interfere with the process of protein formation, which is here again limited by the carbohydrate factor. With high intensities of red and blue-violet rays, the relations between the two processes are similar to those found for daylight and electric light. In the

blue-violet light the formation of proteins takes place normally, while in the red light the process is interfered with by the accumulation of carbohydrates. Greater formation of proteins occurs in the blue-violet light of high intensity than in the same light of low intensity as the carbohydrate supply is increased, but not to the extent of interfering with the process. In the red light of low and high intensities there is no difference in the quantity of protein formed, indicating that the high rate of photosynthesis in the red light of high intensity interferes with the protein formation due to accumulation of carbohydrates, as in daylight.

The results support the conclusion that there is a condition of balance between the two processes occurring in the leaves. During day-time high activity in carbohydrate production loads up the cells with these products and the other process is depressed. The results also show that light has perhaps an indirect role to play in the way of carbohydrate supply in the process of protein synthesis.

SUMMARY

In continuation of the previous work on carbohydrate content of leaves (Dastur et al. 1933, 1935, and 1937) exposed to light of different qualities the water-soluble, organic nitrogen content of leaves of *Helianthus annuus*, *Ricinus communis*, *Abutilon asiaticum*, *Tropaeolum majus*, *Raphanus sativus*, and *Nicotiana Tabacum* have been examined.

The order of increase of the water-soluble N of the leaves in different lights of equal *total* intensity is carbon arc > 'daylight' lamp > ordinary electric lamp > daylight.

If monochromatic light and daylight are compared the organic N content with low light intensity is in the order daylight > red light = blue-violet light. With high intensity, using the carbon arc, the blue-violet is more effective than the red.

The explanation is put forward that during the process of photosynthetic assimilation there is a condition of balance between protein formation and carbohydrate production. When the ratio of the intensity of red to blue-violet ray is high, as in ordinary electric light, the carbohydrate production is depressed and the formation of proteins is limited by the carbohydrate supply. When the ratio is low, as in daylight, the rate of carbohydrate production and the rate of protein formation is depressed by the accumulation of carbohydrates. With an intermediate condition, as in the light of the carbon arc, both processes go on actively, protein formation being more active than in ordinary electric light.

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NOTES

ON THE RELATION BETWEEN THE DISTRIBUTION OF AUXIN IN THE TIP OF THE AVENA COLEOPTILE AND THE FIRST NEGATIVE PHOTOTROPIC CURVATURE.—According to the Cholodny-Went theory the phototropic curvatures in *Avena* coleoptiles are related to the unequal distribution of auxin on the two sides of the coleoptile tip. That actual unequal distribution of auxin does follow on unilateral illumination of the coleoptile tip was experimentally demonstrated for the first time by F. W. Went (*Die Erklärung des phototropischen Krümmungsverlaufs*: 'Rec. trav. bot. néerl.' xxva: 483-9; 1928). He found the difference in the auxin distribution, in the case of first positive phototropic curvature, of the order of 32 and 68 per cent. between the illuminated and dark sides respectively. A detailed discussion of this matter will be found in a recent monograph (Went, F. W. and Thimann K. V., 'Phytohormones', Macmillan & Co., 1937).

When the writer visited the Botanisch Laboratorium, Utrecht during the summer of 1935 it was suggested by Dr. F. W. Went that the question as to whether differential distribution of auxin occurs in the first negative phototropic curvature would be worth investigating. Accordingly this study was undertaken at the Botanisch Laboratorium, Utrecht.

The technique of this type of work is too well known to need any detailed description. It may, however, be recorded that the standard technique employed at Utrecht was strictly followed throughout, and three decapitations, spaced two hours apart, were carried out on test plants, as recommended by Dr. A. N. J. Heyn of the Botanisch Laboratorium, Utrecht.

In order to determine how far auxin distribution was uniform a number of control experiments were carried out on coleoptile tips of plants grown in the dark. The results are as follows:

TABLE I

Curvatures (Degrees) obtained with Test Plants on applying Auxin collected from the Left and Right Sides of Unilluminated Coleoptile Tips. October 1935.

Date.	Left.	Right.	Number of plants.
—	7.55 ± 0.8	8.9 ± 0.82	11
8/10/35	2.90 ± 0.87	3.35 ± 0.71	10
10/10/35	5.68 ± 1.0	5.0 ± 0.82	11
5/10/35	7.75 ± 0.97	6.25 ± 1.48	6
18/10/35	8.23 ± 2.74	8.13 ± 2.75	11
20/10/35	4.54 ± 1.54	5.20 ± 1.77	12
Mean .	6.11	6.14	—

The differences between the means of the individual experiments are not statistically significant according to Student's 't' test. We may thus conclude from the means of six experiments, based on 61 coleoptile tips and 122 test plants in all,

that auxin distribution is equal on the two sides of the coleoptile tip unexposed to light.

It will also appear that the variations from day to day are fairly large. Similar variations have been reported by workers in Utrecht and elsewhere. No satisfactory explanation of this discrepancy has been suggested so far, and for further discussion Went and Thimann's monograph may be consulted.

For the first negative phototropic curvature, the plants were exposed to one-sided illumination, for 40 seconds, from a 350 c.p. lamp at 1 metre distance. The coleoptile tips were then cut off, auxin collected in the agar blocks from the dark and illuminated sides respectively; the tests were carried out in the usual manner and in the same month (Oct. 1935). The results are given in Table II.

TABLE II

Date.	Dark side.	Illuminated side.	No. of plants.	Percentage of control plants showing 1st negative curvature.
11/10/35	3.0±0.11	3.47±0.49	18	—
12/10/35	3.03±0.65	4.14±0.52	17	—
15/10/35	3.97±0.32	3.95±0.29	20	50
17/10/35	7.97±0.97	6.23±0.58	17	64
18/10/35	5.55±1.48	7.75±1.37	10	70
20/10/35	3.87±0.33	5.7±0.75	12	80
24/10/35	5.73±0.92	9.73±0.9	15	64
25/10/35	6.56±0.9	11.12±1.2	18	—
26/10/35	5.00±0.81	8.4±0.77	18	—
28/10/35	3.09±0.52	5.06±0.49	17	60
30/10/35	6.11±0.77	9.72±0.8	18	—
31/10/35	7.77±1.06	11.06±1.09	15	—
Totals .	61.65	86.33	195	—
Means .	5.14	7.19	—	—

It may be pointed out that the differences between the means of the first six experiments are not significant statistically, according to the 't' test, whereas the last six are highly significant. With the exception of experiments performed on 15/10/1935 and 17/10/1935 respectively, all others show greater auxin-content on the illuminated side. The probable reason why the first six results, considered individually, do not attain the level of statistical significance, is that the first negative phototropic curvature is very small as compared to the first positive, and hardly attains a range of 5°; in addition the proportion of plants showing this response is very variable, as will appear from an inspection of column 5 of Table II. On six different occasions about a dozen out of a number of plants exposed for experiment were left with their coleoptiles intact. On no occasion did all the plants show negative response. In the experience of the writer, all the plants exposed for the first positive curvature have without an exception shown very good response. Probably the small range of light intensities in which the first negative curvature occurs, as compared with that for positive curvatures, coupled with the variability of plants, partially accounts for the erratic behaviour during a negative response. Moreover, the percentage of plants showing this response also varies from day to day which makes the study more

difficult. For this reason, the two means of the single experiments have been taken together and two new means calculated and their difference tested for significance both by the 't' and the 'Z' tests. For variance due to treatment 'Z' = 1.426 (theoretical 'Z' for $n_1 = 1$ and $n_2 = 11$ is = 1.133, $P = 0.01$); 'Z' for variance due to different days or occasion = 0.89641 (theoretical 'Z' for $n_1 = 11$ and $n_2 = 11$, is = 0.7785, $P = 0.01$). 't' = 2.2342 (theoretical 't' = 2.07, $P = 0.05$).

The differences due to treatment as well as for occasion of experiment are thus both real and significant.

We may therefore, conclude from these experiments, based on 195 coleoptiles exposed and 390 test plants, that the first negative phototropic curvature is correlated with differential auxin distribution of the order of 42 per cent. on the dark side and 58 per cent. on the illuminated side.

In regard to the question as to whether exposure to light influences the total auxin-production we find that although the mean totals for controls (viz. $6.11 + 6.14 = 12.25$) and for negative curvature experiments (viz. $7.19 + 5.14 = 12.33$) agree very well, it is not safe to draw any conclusion since we have established from the analysis of variance that variance due to occasion is significant; the excellent agreement may, therefore, be quite fortuitous. Only experiments carried out on the same day can decide this point. Data from two such experiments, viz.

Total Auxin Content

Date.	Control.	Negative curvature
18/10/35	16.36	13.30 (not significant)
20/10/35	9.74	9.57 " "

seem to suggest that there is no such effect of light.

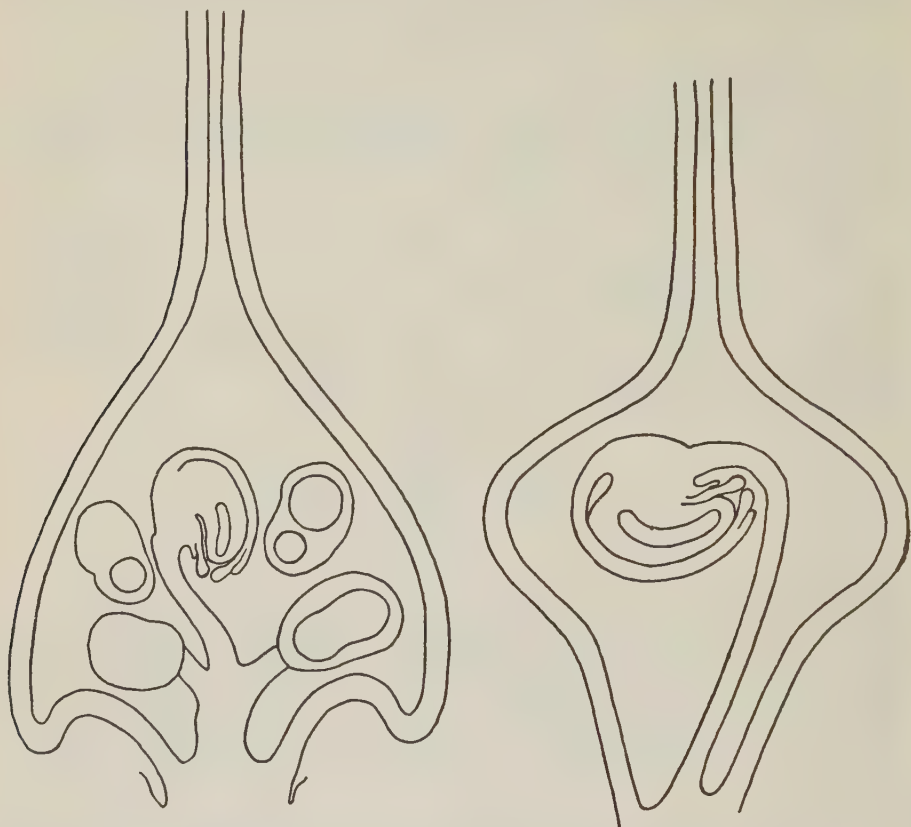
In conclusion the writer wishes to express his thanks to Prof. V. J. Koningsberger, Director of the Botanisch Laboratorium, Utrecht, for generously placing all the excellent facilities of the laboratory at his disposal and for much kindness shown during his stay at Utrecht; and to Dr. F. W. Went for suggesting this problem and for helpful discussion and criticism. The writer's best thanks are also due to other members of the Botanical Staff for unfailing courtesy and much practical guidance.

R. D. ASANA.

THE NATURE OF THE OVULAR STALK IN POLYGONACEAE AND SOME RELATED FAMILIES.—In the Polygonaceae the ovule in most genera is nearly sessile, but in the large-flowered forms like *Brunnichia* and *Coccoloba* it possesses a well-developed stalk. This stalk has been generally called a funiculus as in other angiosperms. R. A. Laubengayer, ('Studies in the Anatomy and Morphology of the Polygonaceous flower', Amer. Journ. Bot., xxiv. 329–43, 1937), however, from his recent studies on the anatomy and morphology of the Polygonaceous flower, comes to a different conclusion. He believes that the single-ovuled gynaecium of this family has been derived by reduction from a many-ovuled condition with free central placentation, and the stalk of the ovule represents a reduced free central placenta; because its vascular supply is comparatively large and the vascular elements are so arranged as to suggest not a single morphological trace, but a product of fusion of the ventral traces of the gynaecium and the ovular traces.

It would have been easy to verify Laubengayer's view as to the exact nature of

the ovular stalk if there were forms in the Polygonaceae with many-ovuled gynaecia. It would then be possible to determine whether the ovules in such types were sessile or possessed a true funiculus. Unfortunately such forms are not to be found. The only method, therefore, of testing Laubengayer's views is to compare the structure



FIGS. 1 and 2. Longitudinal sections of ovaries showing the form and arrangement of the ovules. Fig. 1. *Celosia argentea*. Fig. 2. *Pupalia lappacea*.

of the Polygonaceous ovule with that of related families. Amarantaceae and Chenopodiaceae are two such families. Of these, the former is useful for the present purpose, because it includes some genera with many-ovuled gynaecia, while in the great majority the gynaecium, as in the Polygonaceae, is only one-ovuled. Figs. 1 and 2 illustrate the structure and arrangement of the ovules in this family. *Celosia argentea* Linn. (Fig. 1) is representative of forms with many ovules in the ovary borne on a short free central placenta. *Pupalia lappacea* Juss. (Fig. 2) is representative of uni-cvulate forms. A comparison of the two shows that there is no difference in the structure of the ovule. The stalk of the ovule in the many-ovuled forms is as well developed as in the one-ovuled types. It is therefore of the same nature in the two cases. It is a true funiculus and not a reduced free central placenta except perhaps at the basal extremity.

The study of Amarantaceae thus does not support Laubengayer's conclusion. It appears that in the Polygonaceae also the stalk of the ovule in forms like *Brunnichia* is, for the greater part of its length, a true funiculus. At the most only the basal end which is generally found fused with the ovary wall can be regarded as a vestige of the free central placenta. The unusual vascular supply of the funiculus is to be explained by the fact that the ovule in the Polygonaceae terminates the floral axis. Accordingly, the ventral traces of the carpels necessarily pass into its stalk, just as in the family Leguminosae many axial bundles pass into the solitary carpel.

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AN IMPREGNATION METHOD FOR STAINING STARCH GRAINS.

—Structures in the cell which will not take up stains readily can sometimes be coloured by precipitation of metallic salts. Such impregnation methods are in fairly common use in preparations of animal tissues, but are less often resorted to in botanical microtechnique. A method involving the precipitation of ferric sulphide has been described ('A Plugging Substance in the Vessels of Hops', *Ann. Bot.*, xlii, no. clxviii, pp. 1027 and 1028, Oct. 1928). Similarly, silver iodide can be used for starch grains, rendering them 'brass' yellow (silver nitrate added to tincture of iodine produces a precipitate which as seen under the microscope is of the same colour), or any darker shade desired.

The procedure for this process is as follows: (1) The material should be fixed in acetic alcohol so that the leucoplasts stain well with eosin. (2) A solution of iodine in potassium iodide is left upon the section for about 20 seconds. (3) The section is washed with water, treated with silver nitrate for 20 seconds, and again washed. The process (2 and 3) is repeated. (If desired, the starch grains can be rendered darker by repetition, but in this case their centres are generally of a lighter shade than the rest of the grain. After fifteen repetitions they go nearly black. (4) It is then better to dip the slide into acid hyposulphite of soda for a few seconds only, in order to remove strings of small crystals which otherwise spoil the appearance of the preparation. (If left in this solution for longer, the yellow colour gradually disappears, beginning apparently at the surface and spreading to the centre of the grains.) (5) Haematoxylin is a satisfactory stain for the cell walls and 2 per cent. eosin should be used for ten minutes for the leucoplasts. (The nuclei of the cells also take up the eosin.)

Although leucoplasts are sometimes difficult to find in fixed material, this process is particularly useful for drawing attention to them, because while they are not affected by the deposition of silver iodide their red colour is in good contrast with the yellow starch grains. The method brings out the starch grain laminations, giving to them the appearance of rough 'turning' marks (e.g. in *Phajus grandiflora*).

No success was achieved with the use of other metals which form iodides. Treatment with potassium iodide, in which there is no dissolved iodine, followed by silver nitrate produces no result, while the use of tincture of iodine with this silver salt does give the characteristic yellow colour to the grains. This yellow colour is not

affected by N/10 hydrochloric acid or by strong ammonia. Exposure to light does not appear to have much effect on the colour; in one case a treated section was left in water in bright sunlight for several hours without noticeable change of colour. The method has been tried out on pseudo-bulbs of *Phajus grandiflora*, Pellionia stem, and potato.

Mr. A. G. Dennis of the Biology Department of St. Thomas's Hospital Medical School, assisted in these investigations.

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